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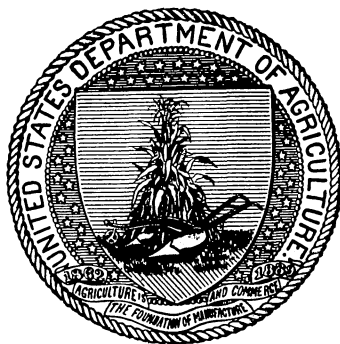
D. E. SALMON, D. V. M., Chief of Bureau.

THE ETIOLOGY OF HOG CHOLERA.

BY

M. DORSET, M. D., B. M. BOLTON, M. D., AND C. N. MCBRYDE, M. D.,

Biochemic Division, Bureau of Animal Industry.



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U. S. DEPARTMENT OF AGRICULTURE,
BUREAU OF ANIMAL INDUSTRY,
Washington, D. C., March 9, 1905.

SIR: I have the honor to transmit herewith for publication, a paper entitled "The Etiology of Hog Cholera," by Drs. M. Dorset, B. M. Bolton, and C. N. McBryde, of the Biochemic Division of this Bureau. The experiments described in this paper were conducted almost wholly with hog-cholera virus obtained from southwestern Iowa. The hog injections and many of the autopsies made in Washington were performed under the supervision of Dr. E. C. Schroeder, Superintendent of the Experiment Station of this Bureau, while all of the injections and autopsies made in Iowa were performed by Dr. W. B. Niles, Inspector.

Very respectfully,

D. E. SALMON,
Chief of Bureau.

Hon. JAMES WILSON, *Secretary.*

INTRODUCTION.

The investigation of hog cholera has been attended with many difficulties, for the reason that the diseases of swine had not been studied with sufficient care to differentiate between a number of diseases liable to be confounded with each other, and because the contagion of hog cholera is not revealed by the ordinary methods of culture and microscopic research. One of the first requisites to the study of any given disease is to be able to recognize that disease with certainty when it is encountered in the field or when it is produced in exposure or inoculation experiments. We supposed it was possible to do this twenty-five years ago, but our recent researches show that the problem was more complicated than we realized at that time. Since the organization of the Bureau of Animal Industry in 1884, I have directed investigations of this destructive plague by every channel of scientific inquiry that gave any promise of results, and I rejoice that I am now able to write the introduction to a bulletin giving in detail the accounts of experiments which throw much light upon the nature of the disease and which explain the obstacles that have impeded our progress in past years.

The discovery of the hog cholera bacillus in 1885 was an important step toward the elucidation of this subject, but it did not prove, as we supposed at the time it would, to be the key to the mystery. The hog cholera bacillus is undoubtedly a disease producing germ. It is uniformly fatal to swine when they are inoculated intravenously, and generally fatal when taken into the digestive canal. It has been found in most cases of the natural disease, and may be recovered from the disease produced by inoculation with cultures. It is extremely fatal to rabbits and guinea pigs by either subcutaneous or intravenous inoculation. The establishment of these facts led to the conclusion that this bacillus was the cause of the disease, but certain discrepancies developed during the course of our investigations which made it almost certain that there was some other factor than this bacillus concerned in the causation of the disease. For instance, there were some outbreaks from which the bacillus could only be obtained with great difficulty, if at all; the animals which recovered from inoculation with the bacillus, although they might be immune from other similar inoculations, were

not immune from the disease as it occurred in the field, but a natural attack of the disease certainly produced immunity; and, finally, it required large doses of the cultivated bacillus to cause disease by subcutaneous inoculation, although an infinitesimal quantity of blood from a diseased pig would cause fatal disease when inoculated in the same manner. It was the great frequency with which the hog cholera bacillus was recovered from outbreaks of the disease in all parts of the country, and its evident disease-producing powers which, notwithstanding these discrepancies, misled us for so many years.

During the years 1893, 1894, 1895, and 1896 Doctor de Schweinitz, by my direction, made a careful investigation of the effects of antitoxic serum in the treatment of the diseases caused by the hog cholera and swine plague bacilli, and he concluded, as a result of his experiments, that these antitoxins had a marked curative influence. In 1897 and 1898 extensive experiments were made in Iowa in the treatment of natural outbreaks of the disease with these serums, which were generally used mixed, as it was not possible to make a definite diagnosis in the field between the diseases caused by these two germs. At first very satisfactory results were obtained, from 75 to 80 per cent of the animals in the infected herds being saved, but later it was recognized that there were very virulent outbreaks of what appeared to be the same disease but upon which the serums had not the slightest influence. This unexpected discovery directed our attention once more to the discrepancies which had previously been remarked with reference to the behavior of hog cholera bacilli when considered as the causative agent of the disease, and I encouraged Doctor de Schweinitz to pursue his investigations and determine, if possible, if there was any other etiological factor concerned in the production of the disease which we were in the habit of calling hog cholera.

After a long and patient investigation, it was decided to issue the circular of September 28, 1903 (Circular No. 41), announcing the most important facts which had been discovered as a result of these researches, in order that other investigators might have the benefit of them. The most striking conclusions published in this circular may be briefly stated as follows:

- (1) There is an infectious disease in this country—not distinguishable from hog cholera—which may be reproduced by infecting material which contains no hog-cholera bacilli.

- (2) This disease is highly contagious by inoculation and by exposure.

- (3) Rabbits and guinea pigs are entirely insusceptible to inoculations that are sufficient to destroy pigs weighing from 30 to 40 pounds.

- (4) The virulent agent of this disease passes through the finest porcelain filters.

On February 12, 1904, a second circular (Circular No. 43) was issued with reference to experiments in the production of immunity, in which

it was stated that "the basis of the immunity experiments has been the use of attenuated and disease-producing liquid or dried blood, or the use of this blood mixed with the blood obtained from immune animals, in which animals the immunity has been increased by the injection of large doses of disease-producing blood obtained from hogs known to have the disease; or, in other words, disease-producing blood and antitoxic blood separate and combined have been successfully used."

The discovery of these facts through the investigations of the Bureau of Animal Industry has removed the obstacles to the further study of hog cholera and pointed out a new field for researches which promise to give us the information by which the plague may be controlled. The death of Doctor de Schweinitz before he was able to prepare an account of the details of his experiments for publication made it necessary for Doctor Dorset and his associates to repeat and extend the experiments. This has been done, and the present bulletin shows their work bearing upon the nature of the disease, which they have performed with the greatest care and accuracy. The researches on immunity are not completed, and the detailed account of the experiments is, therefore, reserved for a subsequent bulletin. It is a source of much gratification to the writer that these successful results have been obtained through the investigations conducted under his direction, and that, notwithstanding the scientific work that has been done with relation to this disease in all parts of the world, we may still say that the principal discoveries have been made in the Bureau of Animal Industry.

D. E. SALMON,

Chief of the Bureau of Animal Industry.

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THE ETIOLOGY OF HOG CHOLERA.^a

By M. DORSET, M. D., B. M. BOLTON, M. D., and C. N. MCBRYDE, M. D.,
Biochemic Division, Bureau of Animal Industry.

PRELIMINARY REMARKS.

After the fundamental work of Salmon and Smith relative to hog cholera, and the corroboration of their observations by so many other investigators, it was regarded as settled beyond dispute that a certain microorganism, *Bacillus cholerae suis*, is the cause of hog cholera. Although certain results of Salmon and Smith, as well as the experience of others, show that it is difficult to reconcile all the facts as found with the acceptance of this bacterium as the exclusive factor in the production of the disease, more or less plausible explanations of these discrepancies have been offered; but these explanations did not seem entirely satisfactory, as will be shown later. Since this is the case, since the etiology is incompletely understood, it is difficult to attack properly the important practical questions of prevention and cure. In the present investigations we believe that, although we have by no means exhausted the subject, we have been able to make some very significant observations which throw light upon the perplexing question, and which we hope may eventually lead to a rational method of combating the disease.

In spite of the fact that the disease hog cholera is so well known, and that it has been so often defined and described in many excellent treatises, it will nevertheless hardly be deemed superfluous to state at the outset just what our experience has taught us to regard as the essential features of the disease, on the one hand, and the variable and inconstant features, on the other. A definite statement in this regard is perhaps particularly called for, since all authorities do not agree upon the relative importance of the different manifestations

^a While attempting to produce an antitoxin for hog cholera by means of injections of cultures of *Bacillus cholerae suis*, Dr. E. A. de Schweinitz, working under the direction of Dr. D. E. Salmon, was led to question the correctness of the prevalent view in regard to the etiology of the disease. He instituted and carried out a number of experiments, most of which were directed toward the discovery of an animal parasite the presence of which he suspected in the blood of sick hogs. His experiments were incomplete, however, and his sickness and subsequent death prevented further research on his part. While he was not actively concerned in the experiments described in this paper, we feel that but for the original skepticism on his part our work would probably not have been undertaken.

seen in the disease. This discrepancy doubtless arises from the fact that in hog cholera, as in other diseases, in different outbreaks and at different times, one or the other set of symptoms and lesions predominate, and it is only by a study of various outbreaks in different parts of the country and over a long period of time that it is possible to arrive at an accurate knowledge of the variations to which the disease is subject.

In the definition and description of the disease given below we have endeavored to present our conception derived from the earlier publications of the Bureau of Animal Industry and from other sources, as well as from the experience we ourselves have had in more recent years.

THE NATURAL DISEASE HOG CHOLERA.

Hog cholera is an acute and highly contagious disease which, so far as known, affects only hogs. It is characterized by fever, loss of appetite, weakness, and, where the affected animal does not quickly succumb, by rapid emaciation. Diarrhea is usually present, though the affected animal may be constipated. Epistaxis frequently occurs, and there is often a gluing together of the eyelids. In the most virulent forms of the disease especially there may be ecchymoses in the skin over the ventral surface of the body. These are usually in the form of discrete splotches, but at times the entire ventral surface is diffusely reddened.

The changes seen in the internal organs vary greatly even in different animals in one and the same outbreak in a given herd. In general these lesions may be said to be either those of a hemorrhagic septicemia or of an ulcerative enteritis, the latter particularly pronounced in the cecum and colon. The hemorrhagic lesions are characteristic of the rapidly fatal form of the disease known as "acute hog cholera," the ulcerative intestinal lesions being especially prominent in those outbreaks where the animals do not succumb so rapidly; both the ulcerative and hemorrhagic lesions may, however, be seen in the same animal. These two forms are not supposed to represent distinct diseases, but merely different types of the same disease, as already stated, the chronic form apparently resulting from either a decreased virulence of the infecting organism or a heightened resistance on the part of the animals which are attacked.

The lesions commonly met with are outlined below.

MORBID ANATOMY.

Subcutaneous areolar tissue.—When the skin of the thorax and abdomen is removed the subcutaneous areolar tissue may be found thickly dotted with ecchymoses of varying size.

Lymphatic system.—In acute hog cholera the inguinal glands on both sides are usually swollen and red, the hemorrhagic condition being so intense at times as to give the glands a bluish black color. The lymphatic glands at the angles of the lower jaw may be affected in a similar manner, as may also the bronchial, mediastinal, mesenteric, mesocolic, retroperitoneal, and lumbar glands. In the chronic form of the disease the lymph glands seldom exhibit any change.

Heart.—This organ frequently presents on its outer surface, and also in the endocardium at times, hemorrhagic markings which vary considerably in their intensity in different cases.

Lungs.—The lungs, as a rule, are but slightly affected. In the acute form of hog cholera they often show ecchymoses of varying size on the serous surfaces; at times areas of broncho-pneumonia or collapse are met with.

Spleen.—In acute hog cholera the spleen, as a rule, is larger than normal, and engorged with blood, and may present numerous punctiform hemorrhages beneath the capsule, or larger hemorrhagic areas which are diffuse in character. In chronic hog cholera the spleen may be smaller than normal, and in this case the connective tissue is noticeably increased.

Digestive system.—(a) *Stomach.* The serous surface of the stomach may be flecked with diffuse hemorrhages, and the mucosa is not infrequently congested and inflamed. This inflammation is at times quite extensive, and may bring about a destructive ulceration of the mucous membrane. Small petechiæ may be seen here and there over the mucous membrane. (b) *Intestines.* In acute hog cholera the chief lesions found are ecchymoses in both serous and mucous coats, together with erosions of the mucous surfaces of both the large and the small bowels. The erosions in the cecum and colon appear to be the starting point of the button-like ulcers which are frequently encountered in the chronic form of hog cholera. These round ulcers vary from 1 to 2 mm. to several centimeters in diameter, and are elevated above the surrounding healthy mucous membrane. They are tough and hard, and their centers are usually dark-greenish yellow in color, and, in the case of the larger ulcers, all four coats of the intestine are involved. The ulcers are at times so numerous as to destroy the mucous membrane, or at least to affect it over extensive areas in the cecum and colon. (c) *Liver.* This organ may exhibit extensive fatty degeneration with areas of coagulation necrosis or an increase of connective tissue. In the acute form of hog cholera, minute hemorrhages may be visible beneath the capsule.

Kidneys.—In acute hog cholera the kidneys are practically always the seat of hemorrhagic changes, which vary more or less in extent. At times the cortices are intensely congested and all of the glomeruli are visible as minute deep-red points. In other instances the general

congestion is absent, the major portion of the kidneys being rather paler than normal, dotted here and there with minute, sharply defined, punctate ecchymoses. At times the medullary portion of the kidneys is involved, and blood clots may be found in the pelvis. In chronic hog cholera these ecchymoses are seldom seen.

Bladder.—Both the serous and mucous surfaces of this organ may be the seat of hemorrhagic changes, which vary considerably in extent in different animals.

In addition to the lesions which have just been described, nearly all of the serous membranes of the body, in the acute form of the disease, may be dotted with hemorrhages. The blood and internal organs of hogs which have died of either acute or chronic hog cholera usually yield pure cultures of a small, motile organism discovered by Salmon and Smith, and named by them *Bacillus cholerae suis*.

Hog cholera may be conveyed from sick to healthy animals almost if not quite without exception by contact, by feeding the viscera of diseased animals, and by the subcutaneous injection of the blood of sick animals. The disease may also be produced, according to Salmon and Smith, by feeding cultures of *B. cholerae suis* and by subcutaneous or intravenous inoculation of that organism. Welch and Clement claim that the disease may be produced by the intratracheal inoculation of healthy animals, as well as by intravenous inoculation with *B. cholerae suis*. Salmon and Smith found it very difficult to produce hog cholera by feeding or by subcutaneous injection of cultures of *B. cholerae suis*, but they consider that in certain instances they succeeded in doing so by both methods.

The mortality among hogs attacked by the disease varies from 30 to 100 per cent of the affected animals, the death rate from the acute type of disease usually exceeding 80 per cent of the affected herd.

THE INFECTIOUSNESS OF THE BLOOD OF ANIMALS AFFECTED WITH HOG CHOLERA.

It will be noticed in the brief description that we have just given of hog cholera that the statement is made that this disease is communicated readily to healthy animals by inoculating them subcutaneously with the blood of hogs suffering with spontaneous hog cholera. The infectious nature of the blood has long been recognized, Law^a having recorded experimental evidence of that fact in 1878. Other experiments showing the infectiousness of the blood of sick hogs are contained in the Bureau of Animal Industry report issued in 1889.^b On page 110 of that report Salmon and Smith describe the inoculation of 4 hogs subcutaneously with the blood of hogs sick of hog cholera, 3

^a Ann. Rept. U. S. Com'r Agr., 1878, p. 377.

^b Hog Cholera: Its History, Nature, and Treatment. Wash., D. C., 1889.

of the 4 inoculated animals dying as a result. We would call attention here to the striking difference between the readiness in producing the disease by the inoculation of blood from animals sick from the spontaneous disease, on the one hand, and the great difficulty experienced in producing the disease by subcutaneous injections of pure cultures of *B. cholerae suis*, on the other. This and other important differences will be fully discussed later.

It is suggested by Salmon and Smith that these successful results may have been due to the greater virulence of the organism in the blood, but they also suggest the possibility that the injected blood coagulating in the tissues protected *B. cholerae suis* from the injurious effects of the body fluids and from the attacks of phagocytes, and at the same time furnished the injected organisms with an abundant supply of suitable food. The bacteria being thus protected for a time might gradually acquire sufficient powers of resistance to enable them to enter the circulation and produce the disease.

At the outset of our present investigations it was deemed wise to repeat Salmon and Smith's experiments with the blood of diseased animals and to ascertain to what the pathogenic power of the blood was due. Accordingly, healthy hogs were inoculated subcutaneously with the blood of sick animals, the infection being derived from a number of separate and distinct outbreaks of hog cholera. We succeeded with the greatest regularity in communicating the disease to healthy animals by the subcutaneous injection of diseased blood obtained during the earlier stages of an outbreak. For convenience the blood was defibrinated previous to injection. It was also found that the clear serum which separated from the red cells in the bottles after the diseased blood was drawn under aseptic conditions possessed the power of inducing the disease in the same manner as the defibrinated blood. In the rare exceptions where the disease was not communicated in this way, it was found that the animal that was injected possessed immunity, as proven by subsequent exposure to infection in a pen with animals sick of hog cholera; there was no exception to this. The subcutaneous injection of blood from diseased animals proved indeed to be such a convenient and reliable method of transferring hog cholera from one animal to another that we have used it almost exclusively for propagating the several strains of disease with which our experiments have been conducted. But while it is true we have experienced no difficulty in thus propagating the disease with inoculations of blood, it should be explained that the different outbreaks which furnished the virus with which we have worked were selected because they appeared to possess a high degree of virulence. It is quite possible that in mild forms of the disease the same difficulty might be experienced in transferring the disease by blood inoculations as is found in transferring it by contact or by any other method.

What has been said above in regard to the result of inoculation with blood applies to blood obtained from animals at the beginning of an outbreak or during its height. Our own experience in propagating the disease by the successive inoculations of a number of hogs during a period of time covering several months has been that after a while the blood inoculations fail to cause the death of the animals. The virus seems to lose in potency after a number of passages through hogs.

The amount of diseased blood required to produce hog cholera when injected subcutaneously has not been definitely determined, but probably varies considerably, depending upon the virulence of the disease and the natural resisting power of the injected hog. We have produced the disease quite uniformly with from $\frac{1}{2}$ to 2 c. c. of blood, and in some instances with much smaller doses. For keeping the disease in existence, larger doses (5 to 10 c. c.) have usually been employed. This was done in order to avoid the loss of the disease through a possible lowering in virulence, the dose in most instances being no doubt much in excess of the amount required.

In the latter part of this bulletin are given the records of many hogs which were injected subcutaneously with the defibrinated blood from diseased hogs containing red blood cells or with the clear serum from such blood. These injections were made for the most part in connection with other experiments, and, although described elsewhere, it seems advisable to present at this point the condensed records of a few of those animals in order that the effect upon healthy hogs of subcutaneous injections of blood from diseased animals may be more clearly brought out.

SUBCUTANEOUS INOCULATIONS WITH BLOOD FROM HERD I.^a

At the time this outbreak occurred there were 23 shoats in the herd, 22 of which died of hog cholera. The history and the lesions found at autopsy were characteristic of acute hog cholera. From a nonimmune hog purposely exposed to this disease, a certain amount of blood was obtained, after the animal had become desperately sick, and used for the injection of hog 650.

Hog 659, injected subcutaneously with 5 c. c. of defibrinated blood from the heart of hog 650 when the latter was quite sick; showed signs of illness 11 days later and 14 days after inoculation, being desperately sick; was etherized in order to obtain blood for experimental purposes.^b The autopsy revealed the lesions of hemorrhagic hog cholera.

Hog 668 was injected subcutaneously with 5 c. c. of the defibrinated heart blood of hog 659. The first symptoms of illness appeared 8 days after inoculation. Fifteen days after inoculation this hog was bleeding from the nose, was hollow in flanks, and very weak, and was killed in order to obtain blood for other experiments. The lesions found at autopsy were petechiæ in the kidneys and under the mucous mem-

^a A more complete description of the outbreaks mentioned here will be found in the latter part of this bulletin.

^b The blood was drawn in this case and in all others, except where otherwise stated, according to the method devised by Dr. E. C. Schroeder, superintendent of the Experiment Station.

brane of the small intestine; in the cecum and colon some ulcers and numerous erosions indicating beginning ulcers.

Hog 689 was injected subcutaneously with 5 c. c. of the defibrinated heart blood of hog 668. The first symptoms of illness were observed 12 days later, and 17 days after inoculation the hog refused food and was apparently very sick. It was, therefore, killed and examined postmortem. Extensive areas of red collapse in the lungs and a few punctate hemorrhages in the kidneys were found.

It might be inferred from the above records, and in particular from the lesions recorded, that this disease became rapidly attenuated. This attenuation was not so rapid as one might infer, however, as in some of the subsequent inoculations the disease reappeared in its original severe hemorrhagic form. In addition, hogs inoculated with blood from those presenting lesions of a mild character became quite as promptly and severely ill as others inoculated with blood from hogs which exhibited extensive hemorrhagic lesions. It is thus evident that the extent of the lesions found at autopsy is not necessarily an index of the degree of infectiousness of the blood.

SUBCUTANEOUS INOCULATIONS WITH BLOOD FROM HERD II.

This herd consisted originally of 20 brood sows and 85 pigs, and as a result of the disease which appeared in June, 1903, more than 92 per cent of the animals died, including 83 of the pigs and 14 of the 20 brood sows. On July 1, 1903, blood was drawn from the heart of one of the sick pigs, defibrinated, and used for the injection of hog 816. The animal which furnished the blood presented at autopsy typical lesions of hemorrhagic hog cholera.

Hog 816 was injected subcutaneously in the manner described above, and showed the first symptoms of illness 8 days later. On the thirteenth day after inoculation the animal was found dead. The autopsy revealed typical lesions of hemorrhagic hog cholera, together with some ulcers in the cecum and colon.

Hog 821 was injected subcutaneously with 5 c. c. of defibrinated heart blood of hog 816. The first symptoms of illness were noted 10 days later. The animal gradually became worse, but did not succumb until 1 month after inoculation. The lesions found at autopsy consisted of many large ulcers in cecum and colon.

Hog 822 was injected subcutaneously with 5 c. c. of the defibrinated heart blood of hog 816. The first symptoms of illness were noted 10 days later. On the eleventh day the hog, being quite sick, was killed to furnish blood for injection of other animals. The autopsy on hog 822 showed large areas of red hepatization in the lungs, a number of small erosions on the mucous surfaces of the stomach, cecum, and colon, and one small ulcer in the cecum.

Hog 846 was injected subcutaneously with 6 c. c. of the defibrinated heart blood of hog 822. The first symptoms of disease appeared 7 days later and, as the hog became worse, it was killed 9 days after inoculation. The autopsy disclosed a few petechial markings on the ventral surface of the body and in the mucosa of the large intestine a number of recently formed ulcers.

Hog 847 was injected subcutaneously with 6 c. c. of the defibrinated heart blood of hog 822. The animal was slightly sick 7 days later and gradually became worse until its death, which took place 17 days after inoculation. The autopsy showed petechiae on the surface of the heart and in the cortices of the kidneys; also several ulcers in the cecum and colon.

SUBCUTANEOUS INOCULATIONS WITH SERUM FROM HERD VIII.

This herd consisted of 536 head of medium-sized hogs when the disease appeared. As a result of the infection, 310 died. On August 12, 1904, a sick hog from this herd was killed. Blood was drawn from the heart, defibrinated, and collected in sterile bottles. On the next day the clear serum which had separated from the corpuscles was drawn off and a portion used for injecting hog 203. The hog from herd VIII, which furnished this blood, exhibited at autopsy

enlarged and hemorrhagic lymph glands, together with beginning ulceration in the large intestine.

Hog 203 was injected subcutaneously with 2.3 c. c. of the clear serum obtained from the defibrinated heart blood of the hog from herd VIII. Previous to injection this serum was diluted with 10 parts of sterile beef broth. The animal showed the first symptoms of illness 15 days later and died 21 days after inoculation. The autopsy disclosed the lesions of acute hemorrhagic hog cholera.

SUBCUTANEOUS INOCULATIONS WITH BLOOD AND SERUM FROM HERD V.

The disease appeared in this herd early in July, 1904, and 103 hogs were exposed to the contagion. The loss occasioned by the disease was 95 per cent of the entire herd. A sick hog from this herd was secured for our experiments. This hog showed at autopsy enlarged spleen, hemorrhagic kidneys, and characteristic ulcers in the cecum.

Hog 184 was inoculated subcutaneously with 2.3 c. c. of clear serum obtained from the defibrinated blood from herd V, mentioned above. The first symptoms of illness were noted 5 days later and the hog (184) died 10 days after inoculation. The autopsy revealed widespread hemorrhagic lesions.

Hog 185 received subcutaneously the same dose of the same material as was used for the injection of hog 184. Five days later hog 185 showed the first symptoms of illness and became gradually worse until death, which took place 18 days after inoculation. The autopsy revealed some hemorrhages in the lymph glands, lungs, and kidneys, together with characteristic ulceration in the cecum.

SUBCUTANEOUS INOCULATIONS WITH BLOOD SERUM FROM HERD VI.

At the time hog cholera appeared there were 76 hogs in this herd, and, as a result of the disease, all but 14 died. A sick hog obtained from the owner of this herd was killed on August 23, 1904, and blood collected directly from the heart. The autopsy revealed hemorrhagic lesions of the internal organs and also a number of ulcers in the cecum and colon. The defibrinated blood was allowed to stand overnight. The next morning the clear serum which had separated was used for the injection of hog 209.

Hog 209 was injected subcutaneously with 2.3 c. c. of blood serum from herd VI, mentioned above, the serum previous to injection being diluted with 10 parts of neutral beef broth. The first symptoms of illness in hog 209 were noticed 11 days later, and death took place 17 days after inoculation. The autopsy revealed the lesions of subacute hog cholera.

TABLE 1.—*Subcutaneous inoculations with blood from various herds.*

No. of hog.	Material injected.	Inoculation.		Autopsy.
		Date.	Result.	
		1903.	1903.	
650	Unfiltered blood, herd I....	Mar. 13	Killed Mar. 31.....	Hemorrhages.
659	do.....	Mar. 31	Sick Apr. 11; killed Apr. 14.	Do.
668	do.....	Apr. 14	Sick Apr. 22; killed Apr. 29.	Hemorrhages and ulcers.
689	do.....	July 29	Sick May 11; killed May 16.	Hemorrhages.
816	Unfiltered blood, herd II....	July 1	Sick July 9; died July 14....	Do.
821	do.....	July 14	Sick July 24; died Aug. 14....	Ulcers.
822	do.....	do.....	Sick July 24; killed July 25....	Do.
846	do.....	July 25	Sick Aug. 1; killed Aug. 3....	Do.
847	do.....	do.....	Sick Aug. 1; died Aug. 11....	Do.
		1904.	1904.	
184	Unfiltered blood, herd V....	July 27	Sick Aug. 2; died Aug. 7....	Hemorrhages.
185	do.....	do.....	Sick Aug. 2; died Aug. 15....	Hemorrhages and ulcers.
209	Unfiltered blood, herd VI....	Aug. 24	Sick Sept. 4; died Sept. 10....	Do.
203	Unfiltered blood, herd VIII.	Aug. 13	Sick Aug. 18; died Aug. 24....	Do.

The above records support fully the statement in the Bureau of Animal Industry report^a that this disease is readily communicated from diseased to healthy animals by the subcutaneous injection of blood from the diseased animals. The injections we have described were made with blood from five distinct outbreaks of hog cholera, and the results indicate, therefore, that the infectiousness of the blood upon subcutaneous injection is a character common to all outbreaks of acute hog cholera. Additional evidence of the infectiousness of the blood of animals suffering from hog cholera is afforded by the results of injections of unfiltered blood in connection with the experiments with filtered blood, described later.

Although we can not speak definitely, owing to lack of experimental data, we would not expect the chronic form of hog cholera to be so readily communicated in this way. The lesions in the chronic form indicate a tendency toward a localization of the infectious centers in the intestines, whereas the acute form possesses the characters of a septicemia.

In addition to proving the ease with which hog cholera may be transferred from one animal to another by the subcutaneous inoculation of blood, the inoculation experiments quoted above indicate that this infectious property is not the result of the mechanical protection of *B. cholerae suis* by the clotted blood, the possibility of which was suggested by Salmon and Smith, as previously stated. All of our blood inoculations were made after the fibrin had been removed, the defibrinated blood containing corpuscles and serum, or the serum alone being used. The serum was diluted with ten volumes of sterile bouillon before injection and could not have afforded any mechanical protection from the phagocytes or other injurious agents. We must therefore look for some other explanation of the fact first observed by Salmon and Smith, namely, that hog cholera may be easily induced by subcutaneous injections of diseased blood, whereas the injections of pure cultures of *B. cholerae suis* in the same manner fail, in the great majority of cases, to produce disease.

THE CONTAGIOUSNESS OF THE NATURAL DISEASE HOG CHOLERA.

In connection with the infectiousness of the blood of hogs affected with hog cholera, the readiness with which transmission of the disease takes place by simple contact with diseased animals, or by exposure in infected pens, must be taken into careful consideration. This high degree of contagiousness of hog cholera is universally recognized by the farmer as well as by those who have made experiments to test the matter; for aside from the abundant evidence of contagiousness to be derived from the records of natural outbreaks of hog cholera, such as

^a Hog Cholera: Its History, Nature, and Treatment. Wash., D. C., 1889.

the accidental introduction of a sick pig into a healthy herd, we would call especial attention to certain records contained in the Bureau of Animal Industry report.^a On pages 145 to 153, inclusive, of that report will be found the records of a number of hogs that had been subjected to various methods of treatment, the object of the treatment being to render them immune from hog cholera. An examination of those records will show that the treated animals and controls were subsequently placed in infected pens to determine the value of the immunizing agent, and that after such exposure to the natural disease, 30 out of a total of 33 that were exposed died of hog cholera. These figures include the check animals which were exposed with the treated ones.

We are in possession of the records of a large number of recent experiments which confirm in every way the evidence cited above, and we would emphasize the importance of this contagiousness as a necessary feature of the disease.

IMMUNITY IN HOGS WHICH HAVE RECOVERED FROM HOG CHOLERA.

Hogs that have recovered from natural attacks of hog cholera are immune from subsequent attacks. This fact is quite generally known among hog raisers and causes the average farmer to place a higher value upon these so-called "cholera proof" hogs than upon nonimmunes. During the course of experimental work in the past few years we have had the opportunity of observing the course of a large number of separate outbreaks of hog cholera, and have tested the immunity of a number of animals that recovered from these natural attacks by means of field exposure and by injections of diseased blood, but have never seen one of them succumb to a second attack. Immunity acquired in this way seems to be remarkably high in degree and very permanent. The same thing has been found to be true of hogs which have recovered from the disease produced by subcutaneous injections of hog-cholera blood, such animals being found immune from hog cholera when exposed to the disease in a natural way and also when injected subcutaneously with blood from diseased animals.

INSTANCES OF IMMUNITY IN HOGS WHICH RECOVERED FROM A NATURAL ATTACK OF HOG CHOLERA.

Hogs 68 and 94: Healthy, untreated shoats. They were first exposed, together with a number of other hogs, on June 12, 1901, to a virulent, spontaneous outbreak of hog cholera. Both animals sickened but subsequently recovered. On August 21, 1901, these hogs, together with a number of nonimmunes, were placed in pens with other hogs which were suffering from a virulent form of hog cholera. Hogs 68 and 94 remained well, and on October 19 were again exposed to natural disease. Both remained well, although the disease was so virulent that nearly 100 per cent of the unprotected animals died.

^a Loc. cit.

Hogs 627 and 629: These two healthy animals were exposed to a natural outbreak of hog cholera July 18, 1901. Both hogs sickened as a result of this exposure, but made a good recovery. They were exposed for a second time on August 21, 1901. Both remained well, and hog 629 was again exposed to diseased hogs on October 19, 1901, but withstood a very severe exposure. Hog 629 was again exposed to hog cholera on our station premises in the summer of 1902 and remained well. After resisting infection in August, 1901, hog 627 was not again exposed until July 23, 1902. On that date this hog was placed with others that were suffering from a natural attack of hog cholera, but did not contract the disease.

Hog 830: This healthy hog contracted a mild attack of hog cholera from some of our experimental animals in April, 1902, but made a complete recovery. On May 29, 1902, it was exposed by being placed with animals sick of hog cholera. The hog did not thrive on this farm, but never became plainly sick.

Hog 772 (a suckling pig): Contracted hog cholera from animals on our station premises in July, 1902, but finally recovered. On October 1, 1902, the pig was placed with hogs suffering from a natural attack of hog cholera. The disease in this herd was very virulent, the mortality among unprotected hogs being almost 100 per cent. Notwithstanding this fact, hog 772 remained well and was exchanged in part payment for the care of our other experimental animals.

Hog 787: Sickened as a result of exposure to hog cholera on the station premises, but recovered; was subsequently exposed, together with hog 772 and other hogs, and showed no sign of illness as a result of this exposure.

INSTANCES OF IMMUNITY IN HOGS WHICH RECOVERED FROM SUBCUTANEOUS INJECTIONS OF HOG-CHOLERA BLOOD.

(Other examples may be found elsewhere in this bulletin.)

Hogs 723 and 724: These animals were inoculated subcutaneously with one-fourth cubic centimeter of defibrinated blood from a sick hog on April 26, 1902. The disease in the herd from which the blood was obtained was quite virulent, and the hog which furnished blood for the inoculation of hogs 723 and 724 exhibited at autopsy the lesions of hemorrhagic hog cholera. These 2 hogs were slightly sick after inoculation, but soon made a complete recovery. On May 29, 1902, they were exposed in the field to acute hog cholera. Neither hog sickened, although unprotected check hogs that were exposed to the same infection died of hog cholera. These 2 hogs, 723 and 724, also survived a subsequent exposure on the station premises.

Hogs 952 and 953: These animals received on October 30, 1902, each one-fourth cubic centimeter of the defibrinated blood of a hog sick of hog cholera. The hogs in the herd presented the usual symptoms and lesions of hog cholera. As a result of the blood injections one of the hogs became sick, but the other showed no visible symptoms of illness. On December 1, both hogs being perfectly well, they were again exposed to hog cholera. All of the checks exposed at the same time died, but hogs 952 and 953 remained well. These 2 hogs were again exposed to a virulent outbreak of hog cholera February 2, 1902. Neither contracted disease as a result of the second exposure.

Hogs 121, 126, 132, and 133: These animals received injections of blood from a sick herd on November 28, 1903. The disease in the herd proved to be of very low virulence, hence it was not surprising that the inoculated pigs showed very little illness after injection. On February 16, 1904, all of the pigs were exposed by being placed with a number of sick hogs. None sickened as a result of this exposure, but it must be noted that the disease to which they were exposed proved to be of low virulence. During July and August, 1904, these animals were again exposed to disease on our station premises. Hog 132 died as a result of this last exposure, but none of the remaining 3 hogs became sick.

Hog 134: This hog was injected with diseased blood December 17, 1903, but showed very little illness as a result, the herd from which the blood was obtained being affected with a rather mild type of hog cholera. On January 2 this pig was reinjected with blood obtained from another sick herd. The pig from which the second supply of blood was obtained presented at autopsy the lesions of subacute hog cholera. No reaction of consequence followed, and on February 16, 1904, the hog was given a field exposure. No sickness followed, and a third exposure in July, 1904, was likewise negative.

Hog 154: This animal, together with hog 155, was injected on May 29, 1904, with blood from a sick herd. Both hogs became very sick as a result of the injection, and hog 155 died. Hog 154 eventually recovered, and was later exposed to virulent hog cholera in our station exposure pen, but did not sicken as a result.

Hogs 161 and 162: Each of these hogs received an injection of blood from a virulent, natural outbreak of hog cholera on June 15, 1904. They both became sick after

injection, but finally recovered and were later placed in the exposure pen, together with a number of other hogs. Both hogs 161 and 162 remained well, although the disease to which they were exposed caused the death of practically all unprotected hogs that were in the pen at the same time.

The above records, together with some which are given in connection with the filtration experiments to be described later, are complete in regard to all the hogs that recovered from the natural disease and that were later tested for immunity.

A point of especial interest which is fairly well brought out is that a severe attack of hog cholera is not necessary for the production of immunity from subsequent attacks. In fact, the protection derived from a mild attack appears to be quite as complete and lasting as that afforded by a severer form of the disease. That this immunity is not transitory is shown by the fact that we have been unable to induce the disease in some of these immunes by injections of diseased blood made nearly a year after recovery from the original attacks.

RECAPITULATION OF THE CHARACTERISTICS OF HOG CHOLERA.

Before proceeding to a description of the various methods we have adopted in our efforts to reproduce hog cholera artificially, we wish to bring together in concise form those characteristics which appear to be essential before any disease may be classified as hog cholera without, however, offering them as a means of differential diagnosis.

(1) The lesions found at autopsy should be those commonly met with in either acute or chronic hog cholera, and which have been already fully described.

(2) The disease should be contagious, as proven by intentional exposure of healthy animals placed in the pen with sick ones or by the history of an outbreak.

(3) The blood of diseased animals should be capable of producing an attack of hog cholera when injected subcutaneously into healthy hogs.

(4) Animals which have recovered from an attack of hog cholera should possess immunity when exposed to natural infection or when injected subcutaneously with the blood of hogs suffering with hog cholera.

The lesions found at autopsy are variable. This variability of the pathological lesions is seen during the course of practically all outbreaks of hog cholera. In some animals there is almost complete absence of lesions; in others there may be few or many of the lesions so generally regarded as pathognomonic of that disease. The hog may be desperately sick, and yet when killed the autopsy may reveal only a few reddened lymphatic glands and possibly a few hepatized areas in the lungs; or, on the contrary, every organ may show pathological changes. Without evidence of contagiousness and without the postmortem examination of other hogs from the same herd, some of

the animals could not be said to have died of hog cholera. On the contrary, the contagiousness, the infectiousness of the blood, and the acquired immunity, after recovery, are very constant features.

EXPERIMENTS WITH PURE CULTURES.

Having become impressed with the constancy of the three characters which were found in all natural outbreaks of acute hog cholera studied by us, and finding after an examination of the literature upon the subject that the questions of the contagiousness of the disease and the infectiousness of the blood in animals injected with pure cultures of *B. cholerae suis* had apparently not been made the subject of experiments in earlier investigations, we determined to make a certain number of experiments which would be in a way supplementary to those already recorded by Salmon and Smith and by Welch and Clement, and which would at the same time enable us to compare the results obtained with the blood and tissues of hogs suffering from hog cholera, on the one hand, with those obtained with cultures, on the other; in short, to see whether hog cholera with the three essential characteristics of contagiousness of sick animals, infectiousness of the blood of sick animals, and immunity when exposed to spontaneous infection after recovery, is found in the disease produced by cultures.

The following experiments were undertaken with a view of obtaining light on these points. In making these experiments some hogs were injected subcutaneously, others intravenously, and others were fed.

SUBCUTANEOUS INJECTIONS OF CULTURES.

In the case of the subcutaneous injections the cultures were in some cases mixed with an equal amount of the defibrinated blood from a normal healthy hog; in other cases the culture alone was used. The addition of the blood was made in order to determine whether normal defibrinated hog's blood possesses any property which would facilitate invasion by *B. cholerae suis*. We were led to make such experiments on account of the difficulty usually experienced in producing disease by the injection of culture alone, whereas injections of defibrinated hog-cholera blood are so uniformly successful.

CULTURE EXPERIMENT NO. 1.

The culture used in this experiment was obtained from guinea pig 4692, which died from the inoculation of 1 c. c. of the undiluted, unfiltered serum of hog 1208, carrying the herd I disease (see Tail-blood Experiment No. 2). A 30-hour bouillon culture, grown at room temperature, was used for the inoculation; this culture was well clouded at the time it was used, but showed only sluggish motility.

TABLE 2.

No. of hog.	Material injected.	Inoculation.		Exposure.		Autopsy.	Remarks.
		Date.	Result.	Date.	Result.		
207	2 c. c. culture + 5 c. c. normal blood.	1904. Aug. 23	1904. Sick Aug. 26; died Sept. 1	1904.	1904.	Hemorrhages and ulcers.	<i>B. cholerae suis</i> present.
214	Exposed to hog 207; not injected.	Exp'd Aug. 27	Sick Sept. 13 to Sept. 17; recovered.	Oct. 6	Remained well.		
217	5 c. c. tail blood hog 207	Aug. 31	Remained well.	do	Died Oct. 15	Ulcerative enteritis	No cultures taken.
220	Exposed to hog 217.	Exp'd Sept. 1	Sick Sept. 4 to Sept. 21; recovered.	Oct. 7	Died Oct. 23	Hemorrhages and ulcers.	No cultures taken.
221	Fed viscera hog 207	Fed Sept. 1					

Hog 207 was injected subcutaneously with 2 c. c. of the 30-hour culture mentioned above, with which was mixed previous to injection 5 c. c. of defibrinated blood obtained from the tail of a healthy hog. Hog 207 was first noticed sick 3 days after inoculation, became rapidly worse, and died 9 days after receiving the injection of blood and culture. The animal had diarrhea, and exhibited the symptoms usually seen in acute hog cholera. Autopsy showed reddening of the skin over pubic region and about nostrils; inguinal glands and lymphatic glands generally enlarged and reddened; lungs congested and also contained scattered hemorrhagic areas; liver much congested, dark in color, and rather firm; spleen greatly enlarged, dark, and very soft; kidneys congested and thickly studded with small punctate hemorrhages; mucosa of stomach toward pylorus much congested; numerous small hemorrhagic splotches on auricular appendages of heart; mucosa of small intestine distinctly hyperemic; mucosa of cecum much congested with numerous small, but well-defined, early ulcerations, covered with a thick yellow, necrotic exudate; mucosa of colon also much congested. Agar cultures from the heart blood and spleen showed pure growths of *B. cholerae suis*.

Hog 214 was not inoculated, but placed in pen with hog 207. The animal was first noticed to be sick in 17 days, with eyes tightly glued together and loss of appetite; remained sick for 3 or 4 days, the eyes being badly affected, but showed rapid improvement and was quite well again in the course of a day or two; was placed in an exposure pen for 7 weeks and remained quite well.^a

Hog 217 was injected subcutaneously with 5 c. c. of defibrinated tail blood of hog 207. This animal remained well in the experiment pen, was transferred to the exposure pen after 36 days, and died 9 days later. Autopsy showed enlarged spleen, mucosa of stomach much reddened, extensive ulceration of cecum and colon. No cultures were taken.

Hog 221 was fed viscera of hog 207, showed symptoms of sickness after 3 days, and was slightly sick for about 2 weeks, but recovered and was placed in the exposure pen after 37 days, and died after an exposure of 16 days. Autopsy showed redness of skin over abdomen, enlarged inguinal glands, congestion of liver, spleen enlarged and dark, hemorrhagic kidneys, extensive ulceration of intestines, with characteristic button ulcers in cecum. No cultures were taken.

Hog 220 was placed in the pen with hog 217 at the time the latter was injected, but remained perfectly well. It was injected intravenously 36 days later with 1 c. c. of a 30-hour bouillon culture from hog 207, and died 3 days later. Autopsy showed marked redness of skin over abdomen; inguinal glands enlarged and hemorrhagic; lungs congested, with a few scattered hemorrhagic areas; liver congested; spleen much enlarged, dark, and soft; kidneys greatly congested with hemorrhagic splotches; mucosa of stomach reddened; numerous small hemorrhagic splotches on both large and small intestines, which show on both the serous and mucous surfaces;

^aBy "exposure pen" is meant a pen containing hogs suffering from the natural disease.

mesenteric glands enlarged and reddened. Agar cultures from heart blood and spleen showed *B. cholerae suis*.

At the end of the description of the experiments with cultures, the results of this experiment are fully discussed and compared with the results obtained in other similar experiments. Interpreted by themselves, some of the results would be apt to be regarded as at variance with all of our other experiments. While we have a few other instances in which hogs died as a result of subcutaneous inoculation of cultures to which normal blood had been added, it was nowhere else observed that a healthy hog associated with a hog made sick in this way contracted disease, as would appear to have been the case with hog 214. The fact that hog 217 was not made sick by the injection of blood from hog 207 would make it improbable that hog 214 had been made sick by simple association, for the injection of blood is a much more severe test of infectiousness than is association. The bearing of the lack of immunity in hogs 217 and 221 upon the question as to whether the sickness in hog 214 could be regarded as hog cholera will be apparent after a moment's consideration, and is fully discussed in connection with the other experiments with cultures.

CULTURE EXPERIMENT No. 2.

In this experiment a 48-hour culture, also from guinea pig 4692, was used. This culture had grown for 30 hours at room temperature (about 22° C.), and 18 hours at an incubator temperature of 33° to 35° C., and was well grown at the time of inoculation, but a hanging-drop preparation revealed only sluggish motility.

One hog was inoculated with the culture alone, 1 with normal defibrinated hog's blood, and 1 with a mixture of the two.

Hog 225 was bled from the tail to furnish the normal blood. The animal was kept under observation and remained perfectly well.

Hog 226 was injected subcutaneously with 5 c. c. of normal blood of hog 225; was kept under observation for 30 days and remained perfectly well.

Hog 227 was injected subcutaneously with 2 c. c. of the culture of *B. cholerae suis*, above described, to which was added previous to injection 5 c. c. of blood from hog 225. This animal was kept under observation for 1 month, and remained well except for a little soreness following the inoculation. It was then placed in an exposure pen and died 18 days later. Autopsy showed enlargement of inguinal glands; areas of congestion and some hepatization in the lungs; spleen much enlarged, dark, and soft; liver congested; kidneys pale; characteristic hog cholera ulceration in the cecum.

Hog 228 was placed in the pen with hog 227 at the time the latter was inoculated, and was kept under observation for 30 days; remained well.

Hog 229 was injected subcutaneously with 2 c. c. of the culture alone. Was kept under observation for 30 days and remained well, except for marked stiffness and soreness for several days after the inoculation; was then placed in exposure pen and died 10 days later. Autopsy showed enlarged inguinal glands; lungs somewhat congested; hemorrhages on auricular appendages of heart; spleen much enlarged, dark, and soft; liver congested; kidneys mottled; mucosa of stomach reddened; extensive ulceration of intestines; mesenteric glands red and swollen.

Hog 232 was not injected, but placed in pen with hog 229 eight days after the latter was inoculated; was kept under observation for 22 days and remained perfectly well; was then placed in exposure pen and died 8 days later. Autopsy showed considerable enlargement of spleen; mucosa of stomach reddened; diffuse ulceration of cecum and small intestine, and small, circular, well-defined ulcers in latter.

TABLE 3.

No. of hog.	Material injected.	Inoculation.		Exposure.		Autopsy.	Remarks.
		Date.	Result.	Date.	Result.		
225	Healthy hog furnishing tail blood.	1904.	1904.	1904.	1904.		Observed for 30 days; remained well. Do.
226	5 c. c. defibrinated tail blood hog 225.	Sept. 7.	Remained well.				
227	5 c. c. defibrinated tail blood hog 225 + 2 c. c. guinea pig 4692 culture.	do.	do.	Oct. 7.	Died October 25.	Ulcers	No cultures made
228	Exposed in pen with hog 227.	Exp'd Sept. 7.	do.				Observed for 30 days; remained well. Do.
229	2 c. c. 4692 culture alone.	Sept. 7.	do.	Oct. 7.	Died October 17.	Hemorrhages and ulcers.	No cultures made.
232	Exposed in pen with hog 229.	Exp'd Sept. 15.	do.	do.	Died October 15.	Ulcers.	

In this experiment the inoculations of *B. cholerae suis* were entirely without effect and did not confer immunity from natural infection, nor did the admixture of normal blood enhance appreciably the virulence of the culture.

CULTURE EXPERIMENT NO. 3.

The culture used for this experiment was obtained from the heart blood of hog 207. (See Culture Experiment No. 1.) A bouillon culture, obtained from a single colony on a sloped agar culture made directly from the heart blood of hog 207, was used for the inoculations.

This culture was grown for 30 hours at a temperature of 30° to 35° C., and was well clouded at the time of inoculation and actively motile. This experiment was conducted in exactly the same manner as the preceding one, but 2 hogs were injected in each case instead of 1.

Hog 233 was bled from the tail to furnish blood for the inoculations. This animal remained perfectly well.

Hogs 234 and 235 were each injected subcutaneously with 5 c. c. of normal defibrinated tail blood of hog 233. They were kept under observation for 32 days and remained perfectly well.

Hog 236 was injected subcutaneously with 2 c. c. of a 30-hour bouillon culture from hog 207; sore and stiff for 2 days after the inoculation. Three days after inoculation, showed symptoms of sickness and remained slightly sick for 4 or 5 days. By the ninth day after inoculation the hog was well again and remained so until the thirtieth day, when it was placed in the exposure pen. Hog 236 remained well in the exposure pen for 40 days, and was removed to other quarters.

Hog 237 was injected in the same manner as hog 236; was sore and stiff for 2 days after inoculation; 3 days later showed symptoms of sickness and was slightly sick from the third to sixth day, inclusive, after inoculation; was reported well 7 days later; from the ninth to thirtieth days after inoculation the hog remained well, and was then placed in exposure pen, where it was kept for 40 days. The animal became sick in exposure pen, but recovered.

TABLE 4.

No. of hog.	Material injected.	Inoculation.		Exposure.		Autopsy.	Remarks.
		Date.	Result.	Date.	Result.		
233	Healthy hog furnishing tail blood.	1904.	1904.	1904.	1904.		Observed for 30 days. Remained well.
234	5 c. c. tail blood hog 233.	Sept. 15.	Remained well.				Observed for 32 days. Remained well.
235	do.	do.	do.				Do.
239	5 c. c. tail blood hog; 233 + 2 c. c. culture.	do.	Sick Sept. 18 to Oct. 10; recovered.	Oct. 20	Died Nov. 9.	Ulcers	No cultures taken.
240	do.	do.	Sick Sept. 18; died Oct. 24.			Necrotic areas in lungs; enlarged spleen; cirrhosis of liver.	<i>B. cholerae suis</i> present.
241	Exposed to hogs 239 and 240.	Exp'd Sept. 15.	Remained well.	Oct. 20	Remained well.		
236	2 c. c. culture.	Sept. 15.	Sick Sept. 18 to Sept. 21; recovered.	Oct. 15	do.		
237	do.	do.	do.	do.	Slightly sick; recovered.		
238	Exposed to hogs 236 and 237.	Exp'd Sept. 15.	Remained well.	do.	Died Oct. 27.	Hemorrhages.	No cultures taken.
245	5 c. c. tail blood hog 239.	Sept. 21.	do.	Oct. 20	Died Nov. 2.	Ulcers	Do.
246	do.	do.	do.	do.	do.	do.	Do.
247	Exposed to hogs 245 and 246.	Exp'd Sept. 21.	do.				Observed 31 days. Remained well.

Hog 238 was placed in pen with hogs 236 and 237. Was kept under observation for 30 days and remained well; was then placed in exposure pen and died 12 days after being exposed to natural disease. Autopsy showed redness of skin; enlargement of inguinal and mesenteric glands; areas of consolidation in lungs; spleen enlarged and dark; liver congested; punctate hemorrhages in the kidneys; small pin-point hemorrhages on mucosa of intestines. No cultures were taken.

Hog 239 was injected subcutaneously with 2 c. c. of the culture from hog 207 with the addition of 5 c. c. of normal tail blood from hog 233. Was first noticed sick 3 days later, and was quite sick from the fifth to tenth days after inoculation; the animal then began to improve, and except for loss in weight, made a good recovery. Was placed in exposure pen 35 days after inoculation, and was found dead 20 days later. Autopsy showed enlarged inguinal glands; extensive areas of consolidation with necrotic centers in lungs, and adhesions between lungs and chest wall; enlarged spleen; cirrhosis of liver; kidneys pale; extensive ulceration of cecum. No cultures were taken.

Hog 240 was injected subcutaneously in the same manner as hog 239; showed first symptoms of sickness 3 days after inoculation and was quite sick from the fifth to the seventeenth day. The animal then showed symptoms of improvement, and for 10 days seemed to improve somewhat in general condition, but grew gradually thinner and weaker and died 37 days after inoculation. In this animal lung symptoms were prominent, the animal showing marked difficulty in breathing. Autopsy showed enlargement of inguinal glands; necrotic areas in lungs, with cavities filled with thick, greenish, cheesy material, extensive pleural adhesions and enlarged bronchial glands; cirrhosis of liver; enlarged spleen. Cultures from the heart blood and spleen showed *B. cholerae suis*.

Hog 241, placed in pen with hogs 239 and 240, was kept under observation for 35 days and remained well; was kept in exposure pen for 48 days and remained well.

Hogs 245 and 246 were each injected subcutaneously with 5 c. c. of defibrinated tail blood of hog 239. These animals were kept under observation for 29 days, and remained well; they were then placed in the exposure pen, and both died 13 days after exposure, having been sick for several days. The autopsies on these animals both showed enlargement of spleen, congestion of lungs, liver, and kidneys; marked hyperemia of stomach mucosa; engorgement of intestinal blood vessels, with enlargement of mesenteric glands. In the case of hog 245 the inguinal glands were enlarged but not hemorrhagic, and the intestinal tract showed ulceration of colon but not of the cecum. In the case of hog 246 there was redness of skin over the abdomen and diffuse ulceration of the cecum and colon, and marked hyperemia of the mucosa of the small intestine. No cultures were made.

Hog 247 was placed in pen with hogs 245 and 246. This animal remained perfectly well and was not exposed.

In this experiment but one animal, hog 240, died as a result of the inoculation. This animal received 5 c. c. of normal blood plus 2 c. c. of culture, and died at the end of 37 days. Hog 239, which received the same inoculation as 240, was made quite sick from the inoculation, but recovered and died subsequently in the exposure pen. Hog 241, the pen check on hogs 239 and 240, remained well in the experiment pen and did not become sick when placed in the exposure pen. In this case it would seem that hog 241 possessed natural immunity, as it showed no signs of sickness as a result of either exposure.

Hogs 236 and 237, which received the culture alone, were both made slightly sick by the inoculation, but recovered. When placed in the exposure pen, hog 236 showed complete immunity, and hog 237 sickened slightly, but recovered. It might appear as if hogs 236 and 237 had acquired immunity from the injection of cultures but for the fact that the majority of our experiments fail to substantiate this interpretation. It should also be noted that these 2 hogs did not communicate disease to their pen check, hog 238. In this experiment the inoculations of blood and culture were apparently more virulent than the inoculations of culture alone, though attempts to reproduce the disease induced by the former were negative.

CULTURE EXPERIMENT No. 4.

The culture used for this experiment was obtained from the heart blood of hog 215 (see Filtration Experiment with herd VIII disease). This culture was obtained as follows: Agar plates were made from the original culture obtained from the blood of hog 215, and showed a pure growth of *B. cholerae suis*. A small flask of neutral bouillon (about 50 c. c.) was then inoculated from a single colony on these plates, and an agar culture was made at the same time from the same colony for final identification. The bouillon culture was incubated for 30 hours at a temperature of 30° to 35° C., and was well clouded and actively motile when used in the following experiment. The agar culture was tested out on the various culture media and found to correspond in all respects with *B. cholerae suis*.

Hog 248, a healthy animal, was bled from the tail to furnish blood for the inoculation of hogs 249, 250, 251, and 252. Was kept under observation for 47 days and remained perfectly well, and was placed in exposure pen at this time and died 16 days later.

TABLE 5.

No. of hog.	Material injected.	Inoculation.		Exposure.		Autopsy.	Remarks.
		Date.	Result.	Date.	Result.		
248	Healthy hog furnishing tail blood.	1904.	1904.	1904.	1904.	Intestinal ulceration.....	No cultures taken.
249	5 c. c. tail blood hog 248	Sept. 21.....	Remained well.....	Nov. 7.....	Died Nov. 23.....	Intestinal ulceration.....	Observed for 31 days; remained well.
250	do.....	do.....	do.....	Nov. 7.....	Died Nov. 14.....	Intestinal ulceration.....	No cultures taken.
251	5 c. c. tail blood hog 248+5 c. c. culture.	do.....	do.....	Oct. 20.....	Died Oct. 31.....	Necrotic enteritis.....	Do.
252	do.....	do.....	do.....	do.....	Died Nov. 7.....	Hemorrhages and ulcers.....	Do.
253	Exposed to hogs 251 and 252	Exp'd Sept. 21.....	do.....	do.....	Died Oct. 31.....	Hemorrhages and necrotic enteritis.....	Do.
254	5 c. c. culture.....	Sept. 21.....	Sick Sept. 22 to Oct. 10; recovered.....	do.....	Died Oct. 29.....	Intestinal ulceration.....	Do.
255	do.....	do.....	do.....	do.....	Died Nov. 8.....	Ulcers.....	Do.
256	Exposed to hogs 254 and 255	Exp'd Sept. 21.....	Remained well.....	do.....	Died Nov. 2.....		Do.

Autopsy showed reddening of skin over abdomen and groins; enlarged inguinal glands; considerable congestion of lungs, with adhesions to chest wall; liver and spleen congested; a few punctate hemorrhages in kidneys; mucosa of stomach congested; extensive ulceration of cecum and colon.

Hog 249 was injected subcutaneously with 5 c. c. of defibrinated tail blood from hog 248; was kept under observation for 31 days, and remained perfectly well.

Hog 250 was injected subcutaneously the same as hog 249 and was kept under observation for 47 days; was then placed in exposure pen and died 7 days later. Autopsy showed inguinal glands enlarged and reddened; liver congested; spleen enlarged and soft; intestinal ulceration.

Hogs 251 and 252 were each injected subcutaneously with 5 c. c. of the culture mixed with 5 c. c. of defibrinated tail blood from hog 248; both were a little sore from the inoculation, but remained otherwise well for 29 days; were then placed in exposure pen. Hog 251 died at the end of 11 days. Autopsy showed considerable congestion and edema of lungs; liver congested; spleen enlarged and dark; kidneys mottled; mucosa of stomach hyperemic; diffuse superficial necrosis of mucosa of cecum and colon. Hog 252 died in the exposure pen at the end of 19 days. Autopsy showed numerous small hemorrhagic areas in the lungs; liver and kidneys congested; spleen enlarged, dark, and soft; extensive ulceration of cecum and colon.

Hog 253 was placed in pen with hogs 251 and 252. This animal was kept under observation for 29 days and remained perfectly well; was then placed in exposure pen and died 11 days later. Autopsy showed some redness of the skin; inguinal glands enlarged; areas of consolidation in lower portions of lungs, and pleura on one side covered with fibrinous exudate; liver congested; spleen enlarged and dark; kidneys much congested, with a few punctate hemorrhages; mucosa of stomach intensely congested; mesenteric glands enlarged and reddened; extensive ulceration of cecum and colon.

Hogs 254 and 255 were injected subcutaneously with 5 c. c. each of the culture alone. Both were quite droopy on the day following inoculation and grew gradually worse. Ten days after inoculation both were quite sick, hog 255 exhibiting a bad case of diarrhea. Both animals then showed signs of improvement, and 9 days later were well again; were placed in exposure pen 29 days after inoculation. Hog 254 died in exposure pen at the end of 9 days. Autopsy showed enlarged inguinal glands; liver congested; spleen enlarged and dark; a few punctate hemorrhages in kidneys; diffuse ulceration of cecum and colon. Hog 255 died in exposure pen at the end of 19 days. Autopsy showed cirrhosis of liver; spleen considerably enlarged; kidneys congested; cecum and colon ulcerated.

Hog 256 was placed in the pen with hogs 254 and 255 at the time these were inoculated; was kept under observation for 29 days and remained perfectly well; was placed in exposure pen at that time and died 13 days later. Autopsy showed enlarged and reddened inguinal glands; lungs, liver, and kidneys congested; spleen greatly enlarged and soft; mucosa of stomach toward pylorus hyperemic; characteristic hog-cholera ulcers in cecum and necrosis of mucosa of colon and small intestine.

In this experiment, contrary to the results obtained in the preceding experiment, the inoculations of culture alone seemed to possess greater virulence than the inoculations of blood and culture.

The inoculations of culture alone made the animals quite sick, but did not confer any immunity from natural infection.

The inoculations of blood and culture had no effect on the animals inoculated and did not confer any immunity from natural infection.

CULTURE EXPERIMENT No. 5.

The culture used in this experiment was obtained from the heart blood of hog 207 (see Culture Experiment No. 1). The bouillon culture was incubated for 30 hours, at a temperature of 30° to 35° C., and was well clouded, and showed active motility when used for the following experiment, which was carried out in exactly the same manner as Culture Experiment No. 3, except that 10 c. c. of culture was injected instead of 2 c. c.

Hog 265 was bled from the tail to furnish blood for the inoculation of hogs 266, 267, 225, and 226. This animal remained perfectly well and was not exposed.

Hogs 266 and 267 were injected subcutaneously with 5 c. c. each of defibrinated tail blood of hog 265. Both animals remained perfectly well and were not exposed.

Hogs 225 and 226 were each injected subcutaneously with 10 c. c. of the culture mixed with 5 c. c. of defibrinated tail blood from hog 265. Both animals were quite sore and stiff as a result of the injection, and were slightly sick for 3 or 4 days. They were placed in exposure pen after 33 days. Hog 225 died in exposure pen after 21 days. Autopsy showed a marked redness of the skin over abdomen; inguinal glands red and swollen; lungs congested; liver congested; spleen large, dark, and soft; kidneys studded with small punctate hemorrhages; extensive ulceration of the cecum with button ulcers; mesenteric glands red and swollen. Hog 226 died in exposure pen after 12 days. Autopsy showed reddening of skin; inguinal glands red and swollen; numerous small areas of congestion in lungs; liver congested; spleen large, dark, and soft; kidneys hemorrhagic; mucosa of stomach hyperemic.

Hog 268 was placed in pen with hogs 225 and 226 at the time these were injected. This animal remained perfectly well in experiment pen and was transferred to exposure pen after 33 days and died 11 days later. Autopsy showed redness of the skin; enlarged inguinal glands; liver congested; hemorrhagic kidneys; mesenteric glands red and swollen; ulceration of the cecum.

Hog 270 was injected subcutaneously with 10 c. c. of the culture alone. The animal was quite sore and stiff for a day or two after the inoculation; was first noticed sick after 5 days and grew gradually worse and died 26 days after receiving the injection of culture. Autopsy showed enlarged inguinal glands; spleen enlarged, dark, and soft; liver and kidneys congested; ulcerations in cecum and colon.

Hog 271 was injected in the same manner as hog 270. The animal was made very sore by the injection and showed marked loss of appetite and slight diarrhea, but soon began to improve and was reported well on the thirteenth day; was placed in exposure pen 33 days after inoculation and died 18 days later. Autopsy showed redness of skin over abdomen; enlarged inguinal glands; congestion of liver; enlarged spleen; ulceration of cecum.

Hog 269 was placed in pen with hogs 270 and 271 at the time these were injected. This animal remained perfectly well for 33 days and was then transferred to exposure pen and died 18 days later. Autopsy showed reddening of skin over groin, inguinal glands and lymphatics generally enlarged and hemorrhagic, lungs and liver congested, spleen much enlarged and soft, a few punctate hemorrhages in kidneys,

TABLE 6.

No. of hog.	Material injected.	Inoculation.		Exposure.		Autopsy.	Remarks.
		Date.	Result.	Date.	Result.		
		1904.	1904.	1904.	1904.		
265	Healthy hog furnishing tail blood.	Oct. 7.	Remained well.	Nov. 6.	Died Nov. 27.	Hemorrhages and ulcers.	No cultures taken.
266	5 c. c. tail blood, hog 265.	do.	do.	Nov. 9.	Died Nov. 21.	Hemorrhages.	Do.
267	do.	do.	do.	do.	Died Nov. 20.	Hemorrhages and ulcers.	Do.
268	5 c. c. tail blood, hog 265 + 10 c. c. culture.	do.	do.	Nov. 9.	Died Nov. 27.	Ulcers.	Do.
269	Exposed to hogs 225 and 226.	Exposed Oct. 7.	Remained well.	do.	do.	Hemorrhages and ulcers.	Do.
270	Exposed to hogs 225 and 226.	Oct. 7.	Sick Oct. 12; died Nov. 2.	Nov. 23.	Slightly sick; recovered.	do.	
271	10 c. c. culture.	Oct. 7.	Sick Oct. 8 to Oct. 20; recovered.	do.	do.	do.	
272	do.	do.	do.	do.	do.	do.	
273	Exposed to hogs 270 and 271.	Exposed Oct. 7.	Remained well.	do.	do.	do.	
274	Exposed to hogs 270 and 271.	Oct. 22.	do.	do.	do.	do.	
275	5 c. c. tail blood hog 270.	do.	do.	do.	do.	do.	
276	do.	do.	do.	do.	do.	do.	
277	do.	do.	do.	do.	do.	do.	
278	do.	do.	do.	do.	do.	do.	
279	do.	do.	do.	do.	do.	do.	
280	do.	do.	do.	do.	do.	do.	
281	do.	do.	do.	do.	do.	do.	
282	do.	do.	do.	do.	do.	do.	
283	do.	do.	do.	do.	do.	do.	
284	do.	do.	do.	do.	do.	do.	
285	do.	do.	do.	do.	do.	do.	
286	do.	do.	do.	do.	do.	do.	
287	do.	do.	do.	do.	do.	do.	
288	do.	do.	do.	do.	do.	do.	
289	do.	do.	do.	do.	do.	do.	
290	do.	do.	do.	do.	do.	do.	
291	do.	do.	do.	do.	do.	do.	
292	do.	do.	do.	do.	do.	do.	
293	do.	do.	do.	do.	do.	do.	
294	do.	do.	do.	do.	do.	do.	
295	do.	do.	do.	do.	do.	do.	
296	do.	do.	do.	do.	do.	do.	
297	do.	do.	do.	do.	do.	do.	
298	do.	do.	do.	do.	do.	do.	
299	do.	do.	do.	do.	do.	do.	
300	do.	do.	do.	do.	do.	do.	

mucosa of stomach intensely reddened, ulceration of the cecum, hemorrhagic patches on heart.

Hogs 247 and 249 were injected subcutaneously with 5 c. c. each of the defibrinated tail blood of hog 270. Both animals remained perfectly well in experiment pen and were transferred to exposure pen on the thirty-second day. After a week in the exposure pen both animals were slightly sick, but soon improved and made complete recovery.

In this experiment but one animal, hog 270, died as a result of the inoculation. This animal received 10 c. c. of culture alone and died after 26 days. Hog 271, which received the same inoculation as 270, was made slightly sick by the injection of culture, but recovered and died subsequently in the exposure pen. Hog 269, exposed in pen with hogs 270 and 271, did not contract the disease and died subsequently in the exposure pen. Hogs 247 and 249, which were injected with 5 c. c. each of the defibrinated tail blood of hog 270, showed no ill effects from the inoculation, and when placed in the exposure pen both animals became slightly sick, but soon recovered. Hogs 225 and 226, injected with blood and culture, were slightly sick from the injection, but died subsequently in the exposure pen.

In this experiment the results agree with those obtained in Culture Experiment No. 4; that is, the inoculations of culture alone seemed to possess greater virulence than the inoculations of blood and culture.

CULTURE EXPERIMENT NO. 6.

Two hogs, 1218 and 1219, were inoculated subcutaneously inside of the thigh with 5 c. c. of a beef-broth culture of *B. cholerae suis* from the heart blood of hog 1190, grown 2 days at 37.5° C. (see herd I disease, p. 55). Both of these hogs remained well after inoculation and were finally transferred to the exposure pen 25 days after inoculation. One of them, 1218, was found

dead in the pen 17 days later; the other one, 1219, was found dead on the twenty-eighth day. Both showed at autopsy the lesions of acute hemorrhagic septicemia and also numerous button ulcers in the colon. Hog 1220, the healthy pen check, which was placed in the experiment pen with these hogs, showed no signs of illness and was removed from the experiment at the time the latter were placed in the exposure pen. The injection of culture caused no illness nor did it confer immunity from natural infection.

CULTURE EXPERIMENT No. 7.

Hog 1454 was injected subcutaneously with 2 c. c. of a bouillon culture from guinea pig 4692. The hog showed some signs of illness 4 days after injection and remained sick for two or three days, but then recovered and was well by the eleventh day. It was then attacked with a diarrhea, but recovered by the thirty-sixth day after inoculation, and was removed to the exposure pen on this date and was found dead 11 days later. The autopsy revealed lesions of hemorrhagic hog cholera.

Hog 1455 was injected subcutaneously with 2 c. c. of the culture from guinea pig 4692, mixed with 5 c. c. of blood from a healthy hog. This animal was placed in a pen with hog 1457, which served as a pen check. Hog 1455 became slightly sick 5 days after the injection, but was entirely well by the eleventh day and remained well for 25 days, when it was removed to the exposure pen, where it was found dead 34 days later. The autopsy showed a large abscess at the site of inoculation. The pleural cavity contained a considerable quantity of turbid fluid; lungs were completely solidified, except the apex of right lung. The lungs were also covered with a thick, tenacious, fibrinous exudate; upon section firm, yellowish white fibrinous areas varying in size from $\frac{1}{2}$ to 1 cm. or more in diameter were found scattered throughout both lungs. There were a few punctiform ecchymoses in the kidneys and spleen. Extensive ulceration of the entire mucosa of the cecum and numerous large button ulcers in the colon. *B. cholerae suis* was obtained in cultures from the organs.

Hog 1457, pen check on hog 1455, remained well for 36 days and was then moved to exposure pen, and was found dead 57 days later. At the autopsy the ventral surface of the body was found to be much reddened; glands at angle of jaw enlarged and sprinkled with many hemorrhagic points; lungs showed extensive hepatization; right lung adherent to chest wall; principal lobes edematous; liver showed many hemorrhagic points; spleen very much enlarged; kidneys showed punctiform hemorrhages in their cortices; small intestines showed many ecchymoses in the mucosa, and in places the mucosa was coated with a thick necrotic exudate; cecum, colon, and rectum contain very many small ulcers.

In the foregoing experiment both the inoculated hogs were made sick but recovered. When exposed later to natural infection, however, both died. The pen check on hog 1455 did not become sick from association with that animal, but died when exposed to natural infection.

INTRAVENOUS INJECTIONS OF CULTURES.

In making the intravenous injections the ear vein was selected in all cases, the ear being first washed with soap and water and shaved, then sponged with 5 per cent carbolic acid, and finally sponged with alcohol; the syringes and needles were sterilized by boiling.

CULTURE EXPERIMENT No. 8.

For this experiment a 24-hour inclined agar culture of *B. cholerae suis* from guinea pig 4692, grown at room temperature, was used. Sufficient sterile, normal salt solution was added to this culture to give, when rubbed up, a suspension approximately equivalent to a 48-hour bouillon culture of *B. cholerae suis* when grown at room temperature.

A healthy hog, 175, was injected in the ear vein with 1 c. c. of this suspension and placed in a carefully disinfected pen along with hog 176 as a pen check. After inoc-

TABLE 7.

No. of hog.	Material injected.	Inoculation.		Exposure.		Autopsy.	Remarks.
		Date.	Result.	Date.	Result.		
189	1 c. c. culture, intravenously	1904. Aug. 1	1904. Sick Aug. 4; died Aug. 30.	1904. Sept. 19	1904. Died Sept. 29.	Intestinal ulceration	<i>B. cholerae suis</i> present.
216	Fed viscera of hog 189	Fed Aug. 30	Remained well.	Sept. 15	Died Oct. 2.	Hemorrhages and ulcers	Do.
206	10 c. c. tail blood, hog 189	Aug. 20	do	Sept. 1	Died Sept. 20.	do	No cultures made.
190	Exposed in pen with hog 189	Exp'd Aug. 1	do	Sept. 1		do	<i>B. cholerae suis</i> present.

ulation the hog was quite sick and refused evening feed; on the following day the animal was very sick, the respirations being hurried, and death occurred a little more than 48 hours after inoculation. Autopsy showed reddening of the skin over axillæ and groins; inguinal glands enlarged, dark, and firm; numerous circumscribed hemorrhagic patches in the lungs; liver dark, with grayish mottling; spleen enlarged, dark, and soft; mucosa of stomach showed patches of congestion toward pyloric end; kidneys swollen, very dark, and presented a coarsely mottled appearance; no intestinal lesions. Agar cultures from heart blood and spleen showed pure growths of *B. cholerae suis*.

Hog 176 was kept in the pen occupied by hog 175 for 16 days, and remained perfectly well.

In this experiment it will be noted that the inoculated animal became sick very soon after inoculation, and this has been our experience generally. There appeared to be no period of incubation in such cases. The uninoculated check did not become sick from association nor from remaining for 16 days in the pen where the inoculated animal had been sick and died.

CULTURE EXPERIMENT NO. 9.

A 48-hour agar culture of *B. cholerae suis* from guinea pig 4692, grown at room temperature, was used for this experiment. To this culture sufficient normal salt solution was added to cause, when rubbed up, a distinct milky cloudiness. A healthy hog, 189, was injected in ear vein with 1 c. c. of this suspension and placed in a disinfected pen with hog 190 as a check.

Hog 189 showed the first symptoms of sickness 3 days after inoculation; 6 days later diarrhea developed and the animal became quite sick; grew gradually worse and died 29 days after inoculation. After diarrhea had set in and the animal was quite sick, on the nineteenth day, blood was drawn from tail for the injection of hog 206. Autopsy on hog 189 showed liver dark, with grayish mottling and apparent thickening of Glisson's capsule; spleen slightly enlarged and dark; kidneys congested, with a few small, bluish black areas; mucosa over pyloric end of stomach reddened; mucosa of cecum showed superficial ulceration near ileocecal valve; lumbar glands were very dark in color. Agar cultures from heart blood and spleen showed *B. cholerae suis*. The greater portion of the viscera of hog 189 was fed to hog 216.

Hog 190, exposed in pen with 189, remained perfectly well for 30 days; was then transferred to exposure pen and died 19 days later. Autopsy showed inguinal glands enlarged and reddened; small, scattered hemorrhagic patches in lungs; liver dark and congested; spleen enlarged, dark, and soft; kidneys congested, with scattered punctate hemorrhages; mucosa over pyloric end of stomach reddened; extensive diffuse ulceration of mucosa of cecum and colon, involving almost the entire mucosa; mesenteric and lumbar glands enlarged and reddened. Agar cultures from heart blood and spleen showed *B. cholerae suis*.

Hog 206 was injected subcutaneously with 10 c. c. of defibrinated tail blood of hog 189 and remained well in experiment pen for 26 days and was then transferred to exposure pen and died 17 days later. Autopsy showed marked redness of skin over abdomen; inguinal glands enlarged and reddened; areas of hepatization in lungs; liver congested; spleen enlarged and dark; kidneys congested, with a few scattered punctate hemorrhages; numerous button ulcers in cecum. No cultures were taken.

Hog 216, fed viscera of hog 189, remained well in experiment pen for 20 days; was then transferred to exposure pen and died 10 days later. Autopsy showed reddening of skin over abdomen; inguinal glands and lymphatics generally enlarged and reddened; areas of consolidation in lungs; spleen slightly enlarged and dark in color; a few punctate hemorrhages in the kidneys; small ulcers in cecum and larger ones in the mucosa of the colon. Agar cultures from heart blood and spleen showed *B. cholerae suis*.

In summarizing the results obtained in this experiment we find that, although hog 189 died as a result of the intravenous inoculation, the disease was not communicated to healthy hogs by contact, by blood injection, or by feeding viscera.

CULTURE EXPERIMENT NO. 10.

For this experiment a 24-hour sloped agar culture from hog 207, grown at a temperature of 30° to 35° C., was used. To this culture 5 c. c. of sterile normal salt solution was added, and the tube thoroughly agitated so as to make a uniform suspension. A microscopic examination of this suspension previous to inoculation revealed an actively motile organism, the suspension appearing under the microscope to be of about the same density as a 24-hour bouillon culture of *Bacillus typhosus*. Two healthy hogs, 242 and 243, were inoculated intravenously with 1 c. c. each of the above suspension and placed in the same pen with hog 244 as a pen check.

Hog 242 was sick on the day following injection, grew rapidly worse, and died 3 days later. The autopsy showed considerable reddening of skin in axillæ and groins; small scattered hemorrhagic areas in lungs; small hemorrhagic splotches on heart; liver dark red and congested; spleen greatly enlarged, dark red, and very soft; kidneys much congested, presenting a dark-red, finely mottled appearance, and, on section, numerous small red points could be seen; mucosa of stomach reddened; inguinal glands and lymphatics generally enlarged and reddened. Agar cultures from heart blood and spleen showed *B. cholerae suis*.

Hog 243 grew rapidly worse, and died the day following injection. The autopsy on this hog showed practically the same lesions as those found in hog 242, except there were no distinct hemorrhagic splotches on heart, and the mucosa of cecum near the ileocecal valve showed a reddened patch. Agar cultures from heart blood and spleen showed *B. cholerae suis*.

Hog 244 was placed in the pen with hogs 242 and 243. This animal was kept under observation in the experiment pen for 31 days and remained perfectly well; was then transferred to exposure pen and died 9 days later. Autopsy showed enlarged inguinal glands, considerable enlargement of the spleen, congestion of liver, and extensive intestinal ulceration. No cultures were taken.

In this experiment the animals that received the intravenous injections of culture both died as a result of the inoculation, with hemorrhagic lesions, and *B. cholerae suis* was recovered from the organs; but the pen check failed to contract the disease.

CULTURE EXPERIMENT NO. 11.

Hog 1295 was injected intravenously with 5 c. c. of a 24-hour beef broth culture of *B. cholerae suis*, from guinea pig 4692; became

TABLE 8.

No. of hog.	Material injected.	Inoculation.		Exposure.		Autopsy.	Remarks.
		Date.	Result.	Date.	Result.		
1295	5 c. c. culture intravenously	1904. June 9	1904. Sick June 10; died June 11.	1904. July 13	1904. Died July 27	Hemorrhages	No cultures taken.
1304	Not inoculated; check on hog 1295	June 11	Remained well.			Hemorrhages and ulcers.	Do.
1312	Fed viscera of hog 1295.	Fed June 11	Sick June 13; killed June 24.			do	<i>B. cholerae suis</i> from liver.
1326	Check on hog 1312.	Exp'd June 15	Remained well.	July 13	Died July 25	do	No cultures taken.
1347	Fed viscera of hog 1312.	Fed June 24	Sick June 27; died July 31.			do	Do.

sick the following day, and died 2 days after inoculation. The autopsy showed extensive hemorrhagic lesions in all the internal organs.

Hog 1304 was placed in the pen with hog 1295 a few hours before the latter died, and remained well; was transferred to the exposure pen 1 month later, where it died at the end of 14 days. The autopsy revealed hemorrhagic lesions and also recent small ulcers at the base of the ileocecal valve.

Hog 1312 was fed with a very large amount of viscera from hog 1295 at the time of death of the latter. The animal, hog 1312, showed signs of illness in 2 days and grew steadily worse for 11 days, when it was killed. The autopsy showed hemorrhagic lesions and *B. cholerae suis* was found in cultures from the different organs.

Hog 1326 was used as a check in the pen with hog 1312. This animal exhibited no signs of illness in the experiment pen, and was transferred to the exposure pen after 1 month, where it became sick and died 12 days later, with hemorrhagic lesions and necrosis of the mucosa of the small intestines.

Hog 1347 was fed large amounts of the viscera of hog 1312 at the time the latter was killed, and at first refused to eat any, but next day consumed all of them. Two days later the animal became sick and continued so until it died, 30 days after being fed viscera. The autopsy showed ulcers in the intestines and also a few hemorrhagic lesions in some of the internal organs, and jaundice in the subcutaneous tissue over the abdomen. No cultures were made.

These results show that association with the hog injected intravenously with the culture did not cause sickness, but that feeding the viscera of the sick hog did cause sickness; also, that this hog in turn did not convey contagion by association, but that the viscera made a hog sick on feeding.

CULTURE EXPERIMENT No. 12.

Hog 1328 was injected intravenously with 1 c. c. of beef broth culture of *B. cholerae suis* from guinea pig 4692. The animal showed signs of sickness immediately after inoculation and grew steadily worse, dying 2 days later.

The autopsy showed the entire ventral surface of the body intensely reddened, the lymphatic glands generally hemorrhagic, and the intestines bound together by a firm network of exudate. The serosa of the intestines was much thickened and opaque, lungs thickly sprinkled with minute ecchymoses, the liver showed ecchymoses, spleen enlarged and congested, mucosa of stomach showed ecchymoses, kidneys intensely congested, mucosa of the cecum and colon showed a number of large congested areas. No cultures were made in this case.

Hog 1337 was injected inside of the left thigh with 5 c. c. of defibrinated blood from hog 1328. The animal showed no distinct signs of illness, but remained stunted in growth, and was further tested, 2 months and 21 days after inoculation, by the injection of 5 c. c. of defibrinated blood of hog 1452, an animal suffering from hog

cholera. Hog 1337 was injured at the time of the injection, September 8, and did not recover from the injury and was found dead 9 days after exposure. The autopsy showed a few hemorrhagic spots in the internal organs, most numerous in the spleen. No cultures were made in this case.

Hog 1338 was fed one-half of the liver, the whole of the spleen, and both kidneys of hog 1328. Three days later this animal was not well, became somewhat worse, but later improved, and finally entirely recovered. While this hog was sick an uninoculated hog, 1346, was placed in the pen with hog 1338 as a check. Hog 1338 was finally placed in the exposure pen after several months and was found dead 5 days later. The autopsy showed a reddening of the glands at the angle of the jaw and some diffuse hemorrhages in the colon, but nothing else worthy of note.

Hog 1346 was placed in the pen with hog 1338, as stated above. This hog remained well, but unthrifty. It was finally placed in the exposure pen, together with hog 1338, and became desperately sick, dying 14 days after exposure to natural disease. The autopsy was very similar to that upon hog 1338, no marked lesions being found.

CULTURE EXPERIMENT No. 13.

Hog 1491 was injected intravenously with 1 c. c. of a dilute suspension of an agar culture from hog 1269. Hog 1491 became sick immediately after this injection, grew rapidly worse and died 4 days after inoculation. The autopsy revealed lesions of hemorrhagic septicemia. *B. cholerae suis* was found in cultures from various organs.

Hog 1492 was injected at the same time with the same dose of the same culture as hog 1491. This animal also became sick at once, grew rapidly worse, and died 4 days after inoculation with the same lesions as found in hog 1491.

Hog 1493, was not inoculated but placed in the pen with hogs 1491 and 1492 as a check. Hog 1493 did not become sick from association with these animals, but remained well for 36 days and was then transferred to the exposure pen and died 27 days later. The autopsy showed a number of hemorrhagic areas in the mucous membrane of the intestines. These were especially numerous in the region of the ileocecal valve.

FEEDING EXPERIMENTS.

CULTURE EXPERIMENT No. 14.

In this experiment a 48-hour bouillon culture of *B. cholerae suis* from guinea pig 4692, grown at room temperature, was used. The culture was well clouded when used for the experiment, but showed only sluggish motility.

Hog 193 was fed 150 c. c. of this culture in a quart of milk and placed in a disinfecting pen with hog 204 as a pen check. Hog 193 was slightly sick on the following day, became rapidly worse, developed diarrhea, and died 12 days after receiving the culture. Autopsy showed lungs slightly congested; liver dark and mottled; spleen dark and soft; mucosa over pyloric end of stomach intensely congested; kidneys congested; large patches of ulceration, covered with a tenacious yellowish exudate in the cecum; diffuse ulceration of mucosa of colon. Agar cultures from heart blood and spleen failed to show *B. cholerae suis*.

Hog 204, exposed to 193, was kept under observation in experiment pen for 29 days and remained perfectly well.

In this experiment the animal fed with culture evidently died as a result of the feeding, but the pen check failed to contract the disease.

CULTURE EXPERIMENT No. 15.

A culture of *B. cholerae suis* obtained from hog 207 was used for this experiment. This culture was obtained as follows: Agar plates were made directly from the heart blood of hog 207 and grown for 7 days at room temperature; the colonies were then well developed and were found to be all similar; a flask of bouillon was inoculated from

one of these colonies and was kept at room temperature (the weather was cool and the laboratory not heated) for 65 hours; at the end of this time the flask was well clouded and furnished the culture used in the following experiment. A microscopic examination of the bouillon culture just previous to feeding revealed a small, actively motile bacillus, and an agar culture made from the same colony as the bouillon culture was tested out on the various culture media and found to correspond in all respects with *B. cholerae suis*.

Hog 231 was fed 150 c. c. of the culture described above in a quart of milk and placed in a disinfected pen; showed symptoms of sickness on the following day, and on the next day had profuse diarrhea and was quite sick; the animal then seemed to improve slightly, but soon grew rapidly worse again and died on the fourth day after receiving the culture. Autopsy showed slight enlargement of inguinal glands; lungs normal; liver dark and mottled; spleen slightly enlarged, dark, and soft; kidneys rather darker than normal with a few small punctate hemorrhages; mucosa of stomach intensely congested; entire mucosa of large intestine and cecum thickened, opaque, and dirty yellow in color—evidently a diffuse necrosis of the mucosa; mucosa of small intestine like that of large intestine, only the condition was not so marked. Agar cultures from spleen showed *B. cholerae suis*.

Hog 204, exposed in pen with hog 231, 2 days after the latter animal was fed culture, remained perfectly well; after 24 days was transferred to exposure pen and died 8 days later. Autopsy showed liver dark and mottled; spleen somewhat enlarged and dark; kidneys congested; diffuse ulceration of cecum, involving the entire mucosa; mucosa of colon also showed diffuse necrosis, but not so extensive as in the case of the cecum. No cultures were obtained from this animal.

In this experiment the animal fed with culture evidently died as a result of the feeding, but the pen check failed to contract the disease and was not rendered immune, as shown by subsequent death in exposure pen.

CULTURE EXPERIMENT No. 16.

The culture used in this experiment was obtained from the heart blood of hog 207 in the following manner: A sloped agar tube was inoculated with heart blood of hog 207 and inclined so as to cause the water of condensation to flow over the surface of the agar. In this culture well-scattered colonies developed, and from one of these colonies a second agar culture was made. From this second agar culture a tube containing 10 c. c. of sterile bouillon was inoculated. The bouillon culture was kept in the incubator at a temperature of 30° to 35° C. for 24 hours, and, when used in the following experiment, the culture was well clouded and actively motile. In carrying out the experiment two healthy hogs, 257 and 258, were each fed 5 c. c. of the above culture in a quart of milk and placed in the same pen. Two days later hog 259 was placed in the pen with these animals as a check.

Hog 257 was slightly sick the day after receiving the culture; grew gradually worse, and died on the ninth day. Autopsy showed liver dark and much congested; spleen enlarged and soft; kidneys congested and mottled; mucosa of stomach very red; mesenteric vessels engorged; mucosa of small and large intestine thickened and ulcerated. Cultures from heart blood and spleen showed *B. cholerae suis*.

Hog 258 was slightly sick the day after receiving culture; became worse, and remained quite sick until the end of 2 weeks, when improvement began. The hog finally recovered and was placed in the exposure pen 27 days after being fed the culture, and died 16 days after exposure. Autopsy showed reddening of skin over

TABLE 9.

No. of hog.	Material used.	Inoculation.		Exposure.		Autopsy.	Remarks.
		Date.	Result.	Date.	Result.		
Pen No. 1. 260	2 c. c. bouillon culture	1904. Fed Sept. 30	1904. Sick Oct. 1; died Oct. 13.	1904.	1904.	Necrotic enteritis	No cultures made.
261	1 c. c. bouillon culture	do	Sick Oct. 1; died Oct. 9	Nov. 6	Died Dec. 1	do	Do.
262	Exposed in pen with hogs 260 and 261.	Exposed Oct. 2.	Remained well.	Nov. 7	Died Nov. 26.	Hemorrhages and ulcers	Do.
Pen No. 2. 263	5 c. c. tail blood of hog 260	Oct. 6.	do	do	Died Nov. 22.	do	Do.
264	do	do	do	do	do	do	Do.

abdomen; inguinal glands and lymphatics generally enlarged and reddened; lungs showed congestion and areas of consolidation; small hemorrhages on auricular appendages of heart; liver congested; spleen enlarged, dark, and soft; numerous small, punctate hemorrhages in kidneys; typical "button" ulcers in cecum and colon.

Hog 259, exposed in pen with hogs 257 and 258, was kept in experiment pen for 21 days and remained well; was then transferred to exposure pen, where it showed evident symptoms of hog cholera—diarrhea, etc. After 10 days this hog was removed to a separate pen and used for another experiment.

In this experiment one of the animals fed with culture died as a result of the feeding, and *B. cholerae suis* was recovered in cultures from the heart blood and spleen; the other animal fed with culture was very sick from the feeding, but recovered and did not acquire any immunity, as shown by subsequent death in the exposure pen. The pen check did not contract the disease and did not acquire immunity.

CULTURE EXPERIMENT No. 17.

A 26-hour bouillon culture of *B. cholerae suis* obtained by plating from heart blood of hog 207, as described in Culture Experiment No. 16, was used in this experiment. The culture was grown at a temperature of 30° to 35° C., and was well clouded and showed active motility. The animals used in the experiment, with brief clinical notes and autopsy records in each case are as follows:

Hog 260, fed 2 c. c. of bouillon culture of *B. cholerae suis* mixed with milk, was slightly sick on the following day; developed diarrhea, and grew gradually worse; died 13 days after receiving the culture. Autopsy showed slight enlargement of inguinal glands; liver congested; spleen enlarged and dark; kidneys mottled; mucosa of stomach reddened; mucosa of small intestine dotted with small, circular, well-defined ulcers; mucosa of cecum and colon thickened and necrotic; mesenteric vessels engorged. No cultures were taken.

Hog 261, fed 1 c. c. of bouillon culture of *B. cholerae suis*, described above, was slightly sick on the following day; became rapidly worse, and died 9 days after receiving the culture. Autopsy showed slight enlargement of inguinal glands; slight congestion of lungs; liver congested; spleen considerably enlarged, dark, and soft; kidneys congested; mucosa of stomach much reddened; mucosa of cecum and colon thickened and necrotic; coronary and mesenteric vessels engorged. No cultures were taken.

TABLE 10.

No. of hog.	Material used.	Inoculation.		Exposure.		Autopsy.	Remarks.
		Date.	Result.	Date.	Result.		
Pen No. 1. 228	$\frac{1}{10}$ c. c. of culture	1904. Fed Oct. 7.	1904. Sick Oct. 8; died Nov. 12.	1904.	1904.	No marked lesions	No cultures made.
272	$\frac{1}{10}$ c. c. of culture	do	Sick Oct. 8 to Oct. 30; recovered.	Nov. 9	Died Nov. 21.	Hemorrhages.	Do.
Pen No. 2. 234	5 c. c. tail blood of hog 272	Oct 17	Remained well	do	Remained well	Hemorrhages and ulcers	Do.
235	do	do	do	do	Died Nov. 23.		

Hog 262, placed in pen with hogs 260 and 261, remained well in the experiment pen; was transferred to exposure pen on the thirty-fifth day and died 25 days later. Autopsy showed enlarged and reddened inguinal glands; lungs congested and pleuræ thickened, with considerable serum in the chest cavity; liver congested; a few punctate hemorrhages in the kidneys; mucosa of cecum ulcerated. No cultures were made.

Hogs 263 and 264 were injected subcutaneously with 5 c. c. each of tail blood from hog 260. Both animals remained well in the experiment pen for 32 days and were then transferred to exposure pen. Hog 263 died in exposure pen at the end of 19 days. Autopsy showed marked reddening of the skin over abdomen; inguinal glands enlarged and reddened; a few small hemorrhagic areas in lungs; liver congested; spleen enlarged; a few punctate hemorrhages in kidneys; mucosa of stomach reddened; numerous small ulcers in cecum. No cultures were taken. Hog 264 died in the exposure pen at the end of 15 days. Autopsy showed a few small hemorrhagic areas in lungs; liver congested; spleen enlarged, dark, and soft; a few small punctate hemorrhages in kidneys; mucosa of stomach reddened; beginning ulceration of mucosa of cecum; mucosa of colon much reddened. No cultures were made.

In this experiment both animals fed with culture died as a result of the feeding. The pen check failed to contract the disease, and did not acquire any immunity, as shown by subsequent death in exposure pen. The two animals inoculated with tail blood of one of the animals fed with culture did not contract the disease and did not acquire immunity, as both died subsequently in exposure pen.

CULTURE EXPERIMENT NO. 18.

A 30-hour bouillon culture of *B. cholerae suis*, obtained from heart blood of hog 207, as described in Culture Experiment No. 16, was used in this experiment. The culture was grown at a temperature of 30° to 35° C., and was well clouded and showed active motility.

The experiment was carried out in exactly the same manner as Culture Experiment No. 17; that is, 2 healthy hogs, 228 and 272, were placed in the same pen and fed separately with bouillon culture in milk. Hog 228 received $\frac{1}{10}$ c. c. of the culture and hog 272 received $\frac{1}{2}$ c. c. Both animals were sick as a result of the feeding, and blood was drawn from the tail of hog 272 on the eleventh day, the animal being then quite sick, and used for the inoculation of hogs 234 and 235, each animal receiving 5 c. c. of the

blood. The animals used in the experiment, with brief clinical notes and autopsy records in each case, are as follows:

Hog 228 was slightly sick for 10 days after feeding. It then improved in condition, but was sick again on the twentieth day, grew gradually worse, and died 36 days after receiving the culture. The autopsy failed to show any marked lesions. No cultures were taken.

Hog 272 was slightly sick on the day after being fed the culture, and continued so for the following week. The animal then seemed rather worse for several days, had no appetite, but soon began to improve and seemed about well 23 days after receiving the culture. The hog was placed in the exposure pen 33 days after receiving the culture, and was found dead 12 days later. Autopsy showed considerable reddening of skin, inguinal glands enlarged and reddened, lungs edematous, liver congested, spleen enlarged and soft, kidneys congested, mucosa over pyloric end of stomach reddened, no intestinal ulceration. No cultures were made.

Hogs 234 and 235 injected subcutaneously with 5 c. c. each of defibrinated tail blood from hog 272; both remained well in the experiment pen, and were transferred to the exposure pen 23 days after inoculation. Hog 234 was kept in exposure pen and remained well. Hog 235 died in exposure pen after 14 days. Autopsy showed marked hemorrhagic lesions, together with diffuse ulceration of cecum and colon. No cultures were made.

In this experiment both animals fed with culture were made sick by the feeding. Hog 228, the animal that received the smaller dose (that is, $\frac{1}{16}$ c. c. of culture), died 36 days after receiving the culture. Whether the death of the animal in this case can be attributed to the culture is hard to determine, as there were no definite lesions at the autopsy. Hog 272, which received $\frac{1}{2}$ c. c. of culture, recovered, but did not acquire any immunity, as shown by subsequent death in exposure pen. Neither of the animals inoculated with tail blood of hog 272 were made sick by the inoculation; one of these animals died in the exposure pen with marked hemorrhagic lesions, the other was immune.

DISCUSSION OF CULTURE EXPERIMENTS.

The results of the subcutaneous inoculation of hogs with cultures of *B. cholerae suis* show that such inoculations produce serious disease in a relatively small number of cases.

Of the 19 hogs injected subcutaneously, only 3 died. Salmon and Smith also encountered this difficulty in producing disease by subcutaneous inoculation, but seemed to regard this as being due to lack of virulence of the organism. This explanation is hardly satisfactory in our cases, for the organisms were very virulent for rabbits and guinea pigs, and produced death in hogs with great uniformity when injected intravenously even in minute amounts, besides producing disease and death when fed to hogs in very small doses. So there can be no doubt as to the virulence of the cultures employed. The fact already brought out, that the injection of blood serum of hog-cholera hogs produces disease promptly and uniformly, suggested the trial of additions of normal defibrinated blood to cultures for subcutaneous inoculation; but the injection of cultures mixed with defibrinated blood proved to be as little efficacious in producing disease as the injection of cultures alone.

The intravenous injection of even very small amounts of culture pro-

duced fatal disease with great regularity, as already stated, and the feeding of cultures frequently caused sickness and death.

In these experiments, then, *B. cholerae suis* was constantly fatal to hogs when administered intravenously, and usually so when administered by mouth, but not so as a rule when injected subcutaneously.

The symptoms produced by the organism when fed are those of intestinal disturbance mainly; the symptoms after intravenous injection are profound depression, loss of appetite, with death in a few days. The autopsy in both cases frequently shows hemorrhagic lesions in the kidneys and other organs, and greater or less necrosis and even ulceration of the mucosa of the intestines, particularly of the cecum in the region of the ileocecal valve, the intestinal lesions being more marked where cultures are fed. So far, then, as symptoms and lesions are concerned, the disease in hogs produced by *B. cholerae suis* may very closely resemble those found in natural outbreaks of hog cholera. At least we are unable to make out any essential differences between the two diseases in these respects. But in certain other very important respects there exists a most striking difference; for, while the disease contracted by natural infection is highly contagious and the blood of animals sick from natural infection is almost always infectious for other hogs by subcutaneous inoculation, and, moreover, while hogs recovered from disease contracted in a spontaneous outbreak possess a high degree of immunity from subsequent infection, all these features are wanting in the disease produced by cultures of *B. cholerae suis*.

The lack of contagiousness in the disease produced by *B. cholerae suis* is rather surprising, for it would be natural to suppose that hogs made sick with the cultures would readily communicate the disease, particularly in those cases accompanied by the profuse diarrhea which is often seen, notably in those where the hogs have been made sick by feeding cultures; yet in only one case—and that not free from objection—is there any indication that healthy hogs contract disease by associating intimately with hogs sick from cultures of *B. cholerae suis*. The hog which became sick while in the pen with a hog sick from the subcutaneous injection of a culture of *B. cholerae suis* is that described above in connection with Culture Experiment No. 1 (p. 14). In this case the symptoms, which developed in 17 days, were those of a passing sickness, shown by diminution of appetite and a gluing together of the eyelids, but all symptoms subsided in 3 or 4 days. This animal afterwards survived exposure in a pen with hogs sick of the natural disease. So, while it can not be asserted positively that this animal was not made sick by contact with the culture hog 207, there are several considerations which make this at least improbable, for a hog, 217, inoculated with blood drawn from the tail of the culture hog 207 remained well after the inoculation and did not acquire immunity, as was shown by the death of the hog in the exposure pen. Again, a hog,

221, fed viscera of hog 207 became somewhat sick from eating the viscera, but also failed to acquire immunity. So, if of the 2 hogs, 217 and 221, the one inoculated with blood and the other fed with the viscera of hog 207, neither became immune, then it is not probable that hog 214 should have acquired immunity from simple association.

There were 21 hogs in all exposed to hogs which were given cultures^a of *B. cholerae suis* by different methods of administration, and hog 214 was the only one of the 21 to show any signs of sickness. Of these 21 hogs exposed to culture hogs, 18 were subsequently placed in a pen with hogs suffering from natural hog cholera, and 16 of the 18 contracted the disease, showing that all except 2 of the 18 hogs were susceptible and would have contracted the disease from the culture animals if these had been suffering from infectious hog cholera, as we understand the disease. Of the 2 hogs remaining well in the exposure pen, one, hog 214, has been discussed above, where it is shown that the animal probably did not derive immunity from the sickness contracted while in the pen with the hog made sick by inoculation with a culture, but in all likelihood possessed natural immunity. The most reasonable explanation of the failure of the other one of these hogs, 241, to become sick in the exposure pen is also that the animal had natural immunity, since it showed no evidences of sickness in the pen with the hogs inoculated with cultures, and one of these, 239, became sick and died in the exposure pen after recovering from the sickness produced by the culture. Hog 240, the companion of hog 239, died from the effects of the inoculation with the culture.

The injection of blood from a hog suffering from the natural infection is uniformly followed by the disease. This is the experience of all who have tested it, and we have abundant experimental proof of this fact, as will be found in the results given on page 18. Indeed, we use this means of propagating the disease for experimental purposes in preference to any other on account of its certainty, as already stated. The injection of blood from animals sick from the administration of cultures, on the contrary, did not produce disease in any of the 11 hogs inoculated in this way. Of these 11 hogs, 10 were exposed to natural infection, 9 became sick, and 7 died. This, again, is in marked contrast with the natural disease, for, in the rare instances of recovery after inoculation with the blood from a hog suffering from hog cholera, there is almost, if not always, without exception, the production of immunity.

Finally, the administration of cultures of *B. cholerae suis* seems to have little, if any, effect upon the production of immunity from natural infection. Incidentally, this has been shown above, in connection with

^a Included in this number are hogs 1326 and 1346, exposed to viscera-fed hogs 1312 and 1338, respectively. (See Culture Experiments Nos. 11 and 12.)

the discussion of the power to produce disease with cultures; but it is worthy of more than passing mention, since it constitutes such a marked contrast with the results following recovery from the spontaneous disease, and from inoculations with blood from hogs suffering from the spontaneous disease.

There were 18 hogs which survived the administration of cultures; 16 of these were injected subcutaneously and 2 fed. Of these 18 hogs 16 died, 1 became sick but recovered, 1 remained well when exposed to natural infection. Of these 2 which survived, both had previously been made sick by subcutaneous injection of cultures. Of the 16 that contracted hog cholera and died in the exposure pen, 6 had recovered after being sick from subcutaneous injection of cultures, 2 from feeding, and 8 had shown no sickness from the administration of cultures.

The difference between the disease produced by *B. cholerae suis* and spontaneous hog cholera, in respect to the immunity of hogs which recover, is very marked; for it can not be admitted that even the hog which failed to become sick in the exposure pen derived its immunity from the administration of cultures, since it may be claimed with equal probability that this animal had natural immunity. Indeed, it seems more probable that the latter explanation is correct in view of the many instances in which illness after administration of cultures failed to produce immunity.

It is clear, therefore, that while the disease produced experimentally with cultures of *B. cholerae suis* corresponds in some respects with spontaneous hog cholera, the two diseases show marked points of difference. They differ in contagiousness, infectiousness, and in the production of immunity from natural infection in animals that recover.

As an explanation of these differences between the natural disease and that produced by *B. cholerae suis*, the possibility that they could be accounted for by a sudden and profound alteration in the disease-producing properties of *B. cholerae suis* occurred to us. But this did not seem probable, for the reason that many cultures employed in the foregoing experiments were tested only a few days after isolation from the body of the sick hog; moreover, these same cultures proved to have retained full virulence for guinea pigs and rabbits after cultivation for several months.

But if these conflicting results between the natural disease and that produced by *B. cholerae suis* are not due to a rapid change in virulence of the organism for hogs they must be due to the presence in the blood of some agent which is absent from the pure cultures, and which, acting either alone or in conjunction with *B. cholerae suis*, is able to bring on a typical attack of hog cholera.

In order to determine the presence or absence of this hypothetical virus in the blood of hogs sick of hog cholera, it became necessary to see whether we could obtain infectious blood from which *B. cholerae suis* was absent.

Two methods of obtaining such blood were finally adopted. The first was based upon the supposition that if two microorganisms were concerned in the production of hog cholera they would probably not always invade the body simultaneously. Hence, by drawing blood from hogs at different stages of the disease we might be able to find a time during the illness when *B. cholerae suis* would be absent and the hypothetical virus would be present in the blood.

EXPERIMENTS WITH DISEASED BLOOD FREE FROM *B. CHOLERÆ SUI*S.

In order to obtain blood from the same hog on a number of consecutive days, we have used a very ingenious method which was devised by Dr. W. B. Niles. In hogs the superficial blood vessels are either so small or are so situated that it is impracticable to use them for obtaining blood by the methods ordinarily employed. Doctor Niles found that by cutting off the end of the tail an abundant supply of blood could be obtained, and, in addition, the blood drawing was comparatively simple and did not cause serious injury to the hog.

The general plan adopted for carrying out these so-called "tail-blood" experiments was as follows: A hog known to be healthy was injected subcutaneously with blood from a sick animal. Beginning 48 hours later, blood was drawn from the tail of the injected hog; a certain amount of the blood obtained in this way was injected subcutaneously into a healthy hog, and an equal quantity placed in neutral beef broth and incubated, the culture being made in order to determine whether or not *B. cholerae suis* was present. The object of these experiments was to determine whether the time at which the blood becomes infectious coincides with the appearance of *B. cholerae suis*, or whether this is independent of the appearance of that organism.

There was one contingency which it was feared might interfere with this experiment. In injecting the original hog with diseased blood the chances were in favor of *B. cholerae suis* being present in the injected blood. Under such circumstances it was feared that this organism would invade the body immediately and thus defeat our object. However, as no better method appeared available at the time, the experiments were conducted according to the plan described above, and it will be seen from the results that our fears were groundless, for *B. cholerae suis* did not appear in the general circulation until several days after the hogs were inoculated. The details and results of these experiments are given in the following pages.

TAIL-BLOOD EXPERIMENT No. 1.

In order to obtain virus for starting this experiment, hog 1103, an animal which had contracted disease by association with others suffering from the natural disease, was etherized when in a desperately sick condition, and blood was drawn from the heart for the inoculation of hog 1149, described below.

The autopsy on hog 1103 showed reddening of skin over ventral portion of body; small hemorrhagic areas in the lungs; numerous petechiæ over auricles; liver mottled, with areas of congestion; spleen enlarged and dark, with areas of apparent necrosis; kidneys swollen and rather pale, with punctate hemorrhages; mucosa of stomach much reddened in places; the small intestine was thickly sprinkled with small hemorrhagic splotches, which appeared on both the serous and mucous surfaces; the mucosa of cecum and colon showed patches of thick necrotic exudate which, when scraped away, revealed shallow ulcers; inguinal glands and lymphatics generally red and swollen. Cultures from heart blood showed *B. cholerae suis* and *B. pyocyaneus*.

In carrying out the experiment, blood was first drawn from the tail of a healthy hog, 1149, under as nearly aseptic conditions as possible, the following procedure being adopted: The tail was first shaved and scrubbed with soap and warm water; it was next dried with alcohol and ether, and sponged off with bichloride solution (1:700), and then wrapped in cotton saturated with the bichloride, which was allowed to remain for 20 minutes; the tail was then washed, first with sterile water and then with alcohol, and cut with a razor sterilized in carbolic acid solution; a wide-mouthed bottle, with a broad-cork stopper, which had been well burned in the flame, was held by an assistant, so that the tail rested on the burnt surface of cork, the tail was then seized by the end with a pair of sterile forceps and cut off usually from $\frac{1}{2}$ to 1 inch from the extremity. The blood which flowed from the wound was collected in a wide-mouthed, sterile measuring glass, and immediately defibrinated by whipping it with a bunch of sterile glass rods.

Five cubic centimeters of the defibrinated tail blood of hog 1149 was injected subcutaneously into hog 1151, and 5 c. c. of the whole blood was added to a flask of sterile bouillon (400 c. c.); this was done in order to be sure that hog 1149 was in a perfectly healthy condition and free from all disease when the experiment was begun. Hog 1149 was then inoculated with 5 c. c. of the defibrinated heart blood of 1103, and, beginning 48 hours after the inoculation, blood was drawn daily from the tail of hog 1149 by the method described above, and used for cultures and for the inoculation of a healthy hog; that is, a flask containing 400 c. c. of sterile beef broth was inoculated each day with 5 c. c. of the whole blood, and at the same time a healthy hog was inoculated with a like amount of the same blood after defibrination.

TABLE 11.

No. of hog.	Material injected.	Inoculation.		Exposure.		Autopsy.	Remarks.
		Date.	Result.	Date.	Result.		
1151	5 c. c. tail blood hog 1149, previous to inoculation with blood of hog 1103	1904. Mar. 30	1904. Sick Apr. 18 to 26; recovered.	1904. May 3	1904. Died May 16	Hemorrhagic	No cultures made.
1153	5 c. c. tail blood hog 1149, 2 days after inoculation with blood of hog 1103.	Apr. 1	Sick Apr. 11 to 26; recovered.	do.....	No effect; killed June 19.	Negative	Do.
1156	5 c. c. tail blood hog 1149, 3 days after inoculation with blood of hog 1103.	Apr. 2	Sick Apr. 11; died Apr. 15	do.....	do.....	Hemorrhages	<i>B. cholerae suis</i> present.
1157	5 c. c. tail blood hog 1149, 4 days after inoculation with blood of hog 1103.	Apr. 3	Sick Apr. 11; died Apr. 14	do.....	do.....	do.....	Do.
1158	5 c. c. tail blood hog 1149, 5 days after inoculation with blood of hog 1103.	Apr. 4	Sick Apr. 11; died Apr. 20	do.....	do.....	Hemorrhages and ulcers	Do.

^aThis sickness was probably not due to the injection.

Hog 1151 was injected subcutaneously with 5 c. c. of defibrinated tail blood of hog 1149 previous to inoculation of the latter animal with blood of hog 1103. Nineteen days later the animal became affected with mange and developed a slight case of diarrhea, with loss of appetite, but within a week seemed quite well except for skin disease; was placed in exposure pen 34 days after inoculation and died 13 days later. Autopsy showed congestion of lungs; hemorrhagic splotches on heart; congestion of liver; spleen slightly enlarged; kidneys congested; mucosa over fundus of stomach hyperemic; mucosa of small intestine much congested; mucosa of large intestine studded with small hemorrhagic splotches. No cultures were taken from this animal. The culture from the blood of hog 1149, on the date this hog was inoculated, failed to show *B. cholerae suis*.

Hog 1153 was injected subcutaneously with 5 c. c. of defibrinated tail blood of hog 1149, 2 days after inoculation of the latter with blood of hog 1103. First symptoms of sickness were noticed on the tenth day, and the animal was very sick for 5 or 6 days, but recovered, and 27 days after inoculation was apparently well; was placed in exposure pen on the thirty-second day, and survived an exposure of 42 days, but remained thin and runty and was killed later. Autopsy revealed extensive hepatization of lungs, and liver pale, with white patches of a fibrous character. No cultures were made from this animal. The culture from the blood of hog 1149 on the date this animal was inoculated remained free from visible growth.

Hog 1156 was injected subcutaneously with 5 c. c. of defibrinated tail blood of hog 1149, 3 days after inoculation of the latter with blood of hog 1103. The first symptoms of sickness were noticed on the ninth day after inoculation, and the animal became rapidly worse and died 4 days later. Autopsy revealed slight reddening of skin over thorax and abdomen and inner aspect of flanks; lymphatic glands generally enlarged and hyperemic; small scattered areas of congestion in lungs; liver dark and mottled; spleen enlarged and dark; kidneys very dark, having a finely mottled appearance, and, on section, the glomeruli showed as hemorrhagic points; mucosa of stomach intensely congested; numerous small splotches on serous surface of small intestine and considerable congestion of mucosa; mucosa of cecum about the ileocecal valve slightly hyperemic. Cultures from heart blood of hog 1156 showed *B. cholerae suis*. The culture from the blood of hog 1149 at the time hog 1156 was inoculated revealed the presence of *B. cholerae suis*.

Hog 1157 was injected subcutaneously with 5 c. c. of defibrinated tail blood of hog 1149, 4 days after inoculation of the latter animal with blood of hog 1103. First symptoms of sickness were noticed on eighth day after inoculation, and the animal became rapidly worse and died 3 days later. Autopsy showed slight

reddening of skin over thorax and abdomen; inguinal glands dark red; small scattered circumscribed areas of congestion throughout both lungs, and almost complete consolidation of median lobe of left lung; petechiæ on epicardium of right ventricle; liver dark and mottled; spleen slightly enlarged and dark; kidneys thickly studded with small punctate hemorrhages; mucosa of stomach intensely reddened, especially noticeable toward pyloric end, and covered with a yellow, tenacious exudate in which particles of food were embedded; slight reddening of mucosa of small intestine; mucosa of cecum and colon dark red in color. Cultures from heart blood of hog 1157 showed *B. cholerae suis*. The culture from the blood of hog 1149 at the time hog 1157 was inoculated revealed the presence of *B. cholerae suis*.

Hog 1158 was injected subcutaneously with 5 c. c. of defibrinated tail blood from hog 1149, 5 days after inoculation of the latter with blood of hog 1103; was first noticed sick on the seventh day after inoculation, grew gradually worse and died 16 days after inoculation. Autopsy showed inguinal glands and lymphatics generally dark red; a few small scattered areas of congestion in the lungs; numerous hemorrhagic splotches on heart, especially noticeable on left auricular appendage; liver rather darker than normal; spleen slightly enlarged and dark, with slightly raised dark-red areas; kidneys thickly studded with small dark-red hemorrhagic points; mucosa over pyloric end of stomach congested; mucosa of small intestine was quite hyperemic; mucosa of cecum dark red, and there were two typical hog cholera buttons near ileocecal valve. Agar cultures from heart blood of hog 1158 showed *B. cholerae suis*. *B. cholerae suis* was present in the blood of hog 1149 at the time hog 1158 was inoculated.

Hog 1149 was injected subcutaneously on March 30, 1904; was first noticed sick 5 days after inoculation, and died on the following day. Autopsy showed slight superficial necrosis at site of inoculation; inguinal glands and lymphatics generally hyperemic; small areas of congestion throughout both lungs; liver dark and mottled; spleen enlarged and dark; almost entire mucosa of stomach intensely congested; mucosa of small intestine slightly hyperemic; mucosa of cecum and colon showed occasional hemorrhagic patches; cultures from heart blood showed *B. cholerae suis*.

The results of the inoculations and cultures from the tail blood of hog 1149 are shown in Tables 11 and 12.

TABLE 12.—Result of cultures from tail blood of hog 1149.

Date of culture.	Source of culture.	Culture medium.	Result of cultures.
1904.			
Mar. 30	5 c. c. tail blood of hog 1149 previous to inoculation with blood of hog 1103.	400 c. c. beef broth	Micrococcus.
Apr. 1	5 c. c. tail blood of hog 1149, 2 days after inoculation with blood of hog 1103.	do	No growth.
Apr. 2	5 c. c. tail blood of hog 1149, 3 days after inoculation with blood of hog 1103.	do	<i>B. cholerae suis</i> .
Apr. 3	5 c. c. tail blood of hog 1149, 4 days after inoculation with blood of hog 1103.	do	Do.
Apr. 4	5 c. c. tail blood of hog 1149, 5 days after inoculation with blood of hog 1103.	do	Do.

In summarizing the results of this experiment we find that the blood of hog 1149 was evidently infectious on the second day after inoculation, as shown by the sickness and subsequent immunity of hog 1151, whereas *B. cholerae suis* did not appear in the blood until the third day.

In addition the virulence of the blood of hog 1149 seems to have increased after the appearance of *B. cholerae suis*. This increase in virulence is not necessarily attributable to *B. cholerae suis*, however, for any other virus concerned in the production of the disease would be expected to increase as the disease progressed toward its height, and it seems not unlikely that the increase in virulence of the blood of

hog 1149 was not wholly dependent upon the presence of *B. cholerae suis*, inasmuch as the appearance of this organism did not perceptibly increase the pathogenic power of the blood in the experiment which follows.

TAIL-BLOOD EXPERIMENT No. 2.

The blood for starting this experiment was obtained from hog 1190, an animal that was sick as a result of an injection described under Filtration Experiment No. 6. A culture from this blood showed a pure growth of *B. cholerae suis*.

Hogs 1208 and 1209 were each inoculated with 5 c. c. of the defibrinated tail blood of hog 1190 and placed in a pen together. Just previous to the inoculation of these 2 hogs blood was drawn from the tail of hog 1208 and 5 c. c. (defibrinated) was injected subcutaneously into healthy hog 1207, and a like amount of the whole blood was added to a flask of sterile bouillon (400 c. c.); this was done in order to make sure that hog 1208 was free from disease when used in the experiment. From hog 1208, beginning 48 hours after inoculation, blood was drawn daily from the tail and cultures and inoculations made as described in Tail-Blood Experiment No. 1. Hog 1209 was allowed to remain undisturbed as a check on the course of the disease in hog 1208.

The following is a list of the animals used in the experiment, with brief clinical notes and autopsy records in each case:

Hog 1207 was inoculated with 5 c. c. of defibrinated tail blood of hog 1208, drawn just previous to inoculation of the latter hog with blood of hog 1190. The cultures from this blood remained free from visible growth. Hog 1207 remained perfectly well, being kept under observation 37 days, when it was placed in exposure pen and died 9 days later. The animal was badly eaten by other hogs in the pen, making an autopsy useless.

Hog 1215 was inoculated with 5 c. c. of defibrinated tail blood of hog 1208, 2 days after inoculation with blood of hog 1190. *B. cholerae suis* was not found in the blood of hog 1208 on this date. Hog 1215 was first noticed sick on the ninth day, and continued very sick for 4 days, when there was slight improvement, but the animal soon relapsed and continued very sick and died 37 days after inoculation. Autopsy showed hemorrhagic lymphatic glands, lungs slightly congested, liver dark and mottled, spleen dark, but not enlarged; kidneys congested, mucosa of stomach over pyloric end much congested, mucosa of both large and small intestine slightly congested, intestines bound down by adhesions, and considerable pale yellow fluid in abdominal cavity. Cultures from heart blood were negative; cultures from liver showed *B. cholerae suis*.

Hog 1216 was inoculated with 5 c. c. of defibrinated tail blood from hog 1208, 3 days after inoculation of the latter hog with blood of hog 1190. *B. cholerae suis* was not found in the blood from hog 1208 on this date. Hog 1216 was first noticed sick 11 days after inoculation and remained so until the twenty-third day; the animal then showed signs of improvement, but remained very thin, weak, and runty, and was killed 43 days after inoculation in order to give room for other experiments. Autopsy showed inguinal glands somewhat enlarged and dark; lungs, liver, and spleen apparently normal; kidneys rather dark and mottled; mucosa of stomach toward cardiac end slightly reddened. Agar cultures from heart blood and kidneys were negative.

Hog 1217 was inoculated with 5 c. c. of defibrinated tail blood of hog 1208, 4 days after inoculation of the latter with blood of hog 1190. A bacillus related culturally to *B. cholerae suis* and *B. coli*, but identical with neither, was found in the blood of hog 1208 on this date. Hog 1217 was first noticed to be sick 14 days after inoculation and continued so until the twenty-second day. The animal then improved somewhat, but suffered a relapse and died 34 days after inoculation. Autopsy

TABLE 13.

No. of hog.	Material injected.	Inoculation.		Exposure.		Autopsy.	Remarks.
		Date.	Result.	Date.	Result.		
1207	5 c. c. tail blood hog 1208, previous to inoculation with blood of hog 1190.	1904. May 2	1904. Remained well.	1904. June 8	1904. Died June 17.	No autopsy.	No cultures made.
1215	5 c. c. tail blood hog 1208, 2 days after inoculation with blood of hog 1190.	May 4	Sick May 13; died June 10.			Hemorrhagic glands; intestinal adhesions.	<i>B. cholerae suis</i> present.
1216	5 c. c. tail blood hog 1208, 3 days after inoculation with blood of hog 1190.	May 5	Sick May 16; killed June 17.			No marked lesions.	No growth from organs.
1217	5 c. c. tail blood hog 1208, 4 days after inoculation with blood of hog 1190.	May 6	Sick May 20; died June 9.			Hemorrhages.	<i>B. cholerae suis</i> present.
1221	5 c. c. tail blood hog 1208, 5 days after inoculation with blood of hog 1190.	May 7	Sick May 16; killed June 17.			Hemorrhagic glands; necrotic areas in liver.	No growth from heart blood.

revealed considerable reddening of the skin over abdomen and groins; lungs congested, with a few scattered, circumscribed hemorrhagic areas; liver congested and lobules showed as hemorrhagic points; spleen enlarged and dark, with numerous large, dark-red, slightly raised areas; kidneys dark and congested and thickly studded with small punctate hemorrhages; extensive ulceration of mucosa of stomach, the mucosa being partially denuded, dark red, and covered with a thick tenacious exudate in which particles of food were embedded; small punctate hemorrhages in small intestines; small red splotches on serous surface of cecum, which extend through to mucosa; lumbar, mesenteric, and mediastinal glands congested. Cultures from heart blood and spleen showed *B. cholerae suis*.

Hog 1221 was inoculated with 5 c. c. of defibrinated tail blood of hog 1208, 5 days after inoculation of the latter with blood of hog 1190. *B. cholerae suis* was found in pure culture in the blood of hog 1208 on this date. Hog 1221 was first noticed sick on the ninth day after inoculation, and remained quite sick for the following 2 weeks; the animal then seemed to improve slightly, but being thin, weak, and runty, was killed on the forty-first day to make room for other experiments. Autopsy revealed reddening of skin in axillæ and groins; lungs slightly congested; liver dark and mottled, with large, yellowish, nodular masses filled with thick greenish yellow pus; spleen normal; kidneys small and rather pale; mucosa of stomach toward fundus much congested; intestinal mucosa also much congested; mediastinal, mesenteric, and lumbar glands dark red and swollen. Agar cultures from heart blood showed no growth.

Hog 1208 was inoculated with 5 c. c. of defibrinated heart blood of hog 1190. The animal was first noticed sick on the seventh day, became rapidly worse and was etherized 3 days later, when in a desperately sick condition. Autopsy showed tissues at site of inoculation slightly indurated and reddened; lungs slightly congested; liver pale, and lobules indistinct, except in places where they showed as small red points; spleen dark, but apparently not enlarged; kidneys pale. Agar cultures from heart blood and spleen showed *B. cholerae suis*.

Hog 1209 was inoculated with 5 c. c. of defibrinated heart blood of hog 1190. The hog was first noticed sick on the seventh day, grew rapidly worse, and died 8 days after inoculation. Autopsy showed inguinal glands on left side and lymphatic glands generally swollen and congested; lungs much congested, with patches of consolidation; liver pale and mottled, and in places the lobules

showed as small red points; hemorrhagic splotches on abdominal surface of diaphragm; spleen enlarged and dark; kidneys congested, with punctate hemorrhages; mucosa over pyloric end of stomach congested; small intestines thickly studded with small punctate hemorrhages, which showed on both the serous and mucous surfaces. Mucosa of cecum and colon much congested, with small erosions in the colon. The cultures from heart blood showed *B. cholerae suis*.

The results of the inoculations and cultures are shown in Tables 13 and 14.

TABLE 14.—Result of cultures from tail blood of hog 1208.

Date of culture.	Source of culture.	Culture medium.	Result of cultures.
1904.			
May 2	5 c. c. tail blood of hog 1208, previous to inoculation with blood of hog 1190	400 c. c. beef broth ...	No growth.
	4 5 c. c. tail blood of hog 1208, 2 days after inoculation with blood of hog 1190.	do	Micrococcus. ^a
	5 5 c. c. tail blood of hog 1208, 3 days after inoculation with blood of hog 1190.	do	No growth.
	6 5 c. c. tail blood of hog 1208, 4 days after inoculation with blood of hog 1190.	do	<i>B. coli communis</i> (?). ^b
	7 5 c. c. tail blood of hog 1208, 5 days after inoculation with blood of hog 1190.	do	<i>B. cholerae suis</i> .

^a No doubt an accidental contamination from the air.

^b This culture showed a small, actively motile bacillus, which produces an acid reaction in and complete coagulation of litmus milk, ferments all three sugars, but does not produce indol.

In summarizing the results obtained in this experiment we find that the blood of hog 1208 was evidently infectious as early as the second day after inoculation, whereas *B. cholerae suis* did not appear in the blood until the fifth day. There was found in the blood of hog 1208 on the fourth day, however, a microorganism which in motility corresponded with *B. cholerae suis*, but which agreed with *B. coli communis* in most of its cultural characters, as previously noted. It may be perhaps significant, though we would not be inclined to lay too great stress upon the fact from the one observation, that this organism appeared in the blood of hog 1208 just before *B. cholerae suis*, and it therefore seems advisable to insert here a brief description of its biological characters.

CULTURE OBTAINED FOUR DAYS AFTER INOCULATION.

A short, thick bacillus with rounded ends, slightly larger than the average *B. cholerae suis*. This organism was very actively motile, the movement being rapid and darting and slightly wriggling in character, though a good many of the organisms in a given field did not move. The indol reaction was negative. On litmus milk a well-marked acid reaction occurred at 1 day, and at 1 week there was complete coagulation with acid reaction. The fermentation test at 72 hours showed fermentation of all three sugars with gas, as follows: Glucose, 30 per cent; lactose, 10 per cent; saccharose, 45 per cent. On neutral beef broth, a very delicate beginning pellicle was noted at the end of 24 hours. This organism was without pathogenic power for guinea pigs. The culture of *B. cholerae suis*, obtained from the blood of hog 1208 on the following day, killed a guinea pig promptly.

SUMMARY OF RESULTS.

In the first experiment the blood was infectious on the second day, whereas *B. cholerae suis* did not appear till the third day. In the second experiment the blood was infectious on the second day and *B. cholerae suis* did not appear until the fifth day. Beyond question, then, the blood of hogs suffering from hog cholera may be infectious for other hogs in the absence of *B. cholerae suis*, as is shown from the results given above. Even if we had only the results in these two cases this conclusion would not be unjustifiable, but when it is supported by the evidence of the filtration experiments below, it becomes unavoidable.

We have laid the emphasis which the matter deserves upon the contagiousness of the natural disease as contrasted with the lack of contagiousness in the disease produced by the use of cultures of *B. cholerae suis*, and it would complete these experiments with the tail blood taken from sick hogs *intra vitam* if it could be shown that hogs made sick by the injection of blood devoid of *B. cholerae suis* could convey the disease to other hogs by association; but this matter was overlooked at the time the experiments were conducted. Still, since it will be shown in connection with the filtration experiments that those hogs inoculated with blood free from *B. cholerae suis* suffered from a highly contagious disease, it is but reasonable to suppose that the disease produced by the injection of tail blood, in all other respects resembling that produced by the filtered blood, should correspond with this in contagiousness as well.

EXPERIMENTS WITH FILTERED BLOOD.

In addition to the experiments with the blood drawn from the tail during life, a second plan adopted was the filtration of diseased blood through Berkefeld and Chamberland cylinders. The filtration experiments were in part conducted before the tail-blood experiments; but for a better understanding of the results it has been deemed best to group all experiments of that character together.

It may be well, before entering upon a description of the filtration experiments, to explain briefly the conditions under which they were performed. First of all we would emphasize the fact that the most rigid precautions against accidental infection have been adopted in all cases. All pens had wooden floors. The disinfection of pens and feeding troughs was in every instance most thoroughly carried out; the attendants in feeding the experimental animals never entered the pen nor was any implement used for cleaning the pens without thorough disinfection, both prior to and after use. The large majority of all hogs in these experiments were held in quarantine several weeks, and, where such a quarantine was impracticable, the animals were obtained from farms which were personally known to be free from

disease. As a result of a rigid enforcement of these precautions there have been in the course of our experiments only a very few instances of disease that could be attributed to accidental infection. Any doubtful cases of this character have been left out of our report or else the conditions have been thoroughly explained.

In the foregoing pages it will be noted that we have insisted upon contagiousness as a necessary feature of hog cholera, and yet it will be found that in some of our filtration experiments pen checks were not used, and therefore the contagiousness of the disease in such instances was not established. In explanation of this fact it should be remembered that our conception of the disease hog cholera has been more or less modified by the results of our experiments; hence in the earlier work some details were omitted which were later found to be desirable. In work of this character, even with all possible facilities at our command, we have not failed to encounter difficulty in making each experiment absolutely complete within itself; but when the work is considered as a whole, we believe the sources of error will be found to have been eliminated so far as possible, and the results show very satisfactory uniformity.

The experiments have been made with eight distinct strains of disease. A part of the experiments were conducted at Washington, D. C., and a part at a temporary station in southwestern Iowa. In obtaining virus from natural outbreaks for experimental purposes we have selected those instances of disease which showed unusual virulence and which would be classed as "acute hog cholera." Preceding each series of experiments will be found a short description of the outbreak from which the virus was obtained originally. The filters used were Berkefeld laboratory cylinders Nos. 5 and 7 (normal) and "Filtres Chamberland, Système Pasteur," marked "F" and "B," and all filtrations were, of course, conducted with sterile apparatus.

The serum was drawn off with a sterile pipette after the blood had stood for 24 hours or more in the refrigerator. The actual amount of serum used in most cases was 50 c. c., though in some cases a smaller amount of serum was used, as it was not always possible to obtain 50 c. c. free from red blood cells. The serum was then diluted with neutral beef broth or physiological salt solution in the proportion of 1 part of serum to 10 parts of the diluent, as stated in each case. The dilution was made for the reason that experience had taught us that the undiluted blood serum filtered very slowly, and in order to facilitate the passage of any virus that might be present. In most cases a fresh bouillon culture of about 4 or 5 c. c. of *B. prodigiosus* was added to test the efficiency of the filtration. The vacuum employed varied from 22 to 25 inches, and the time of filtration was usually very short, about a half hour or less, though at times it was much longer on account of the presence of red blood cells. The apparatus was arranged in some cases

so that the filtration took place from within the cylinder outward, as shown in plate 2; in other cases from without inward, as in plate 3.

The hogs used in the experiments were young animals weighing from 20 to 40 pounds, and the injections were made inside the thighs, using 22 c. c.—11 c. c. on each side—equivalent to 2 c. c. of undiluted serum, except where otherwise stated.

EXPERIMENTS WITH BLOOD FROM HERD I.

About the middle of February, 1903, the animals in this herd, which was located in southwestern Iowa, began showing symptoms of illness. The farm was visited by Dr. Charles M. Day, who reported that the antemortem symptoms were those of hog cholera. Postmortem examinations of 2 of the sickest hogs revealed the lesions of acute hemorrhagic hog cholera. As a result of this outbreak 22 out of 23 hogs died.

Blood from a sick animal in this herd was injected subcutaneously into hog 650, the latter animal being at once shipped to Washington. This hog, 650, served as the starting point for a large number of experiments. By successive subcutaneous inoculations of blood the disease was kept in existence for many months, no difficulty being experienced in transferring the disease to healthy animals in this manner, and the autopsies upon hogs which died from these injections were quite typical of hog cholera.

FILTRATION EXPERIMENT No. 1.

A healthy hog, 704, was inoculated subcutaneously inside the left thigh with 4 c. c. of defibrinated blood from hog 689, and this hog was sick, at the time the blood was drawn, as a result of an injection of 5 c. c. of diseased blood from a hog belonging to herd I.

Hog 704 remained well until 6 days after inoculation, when the first symptoms of illness were observed, and 3 days later, when the hog was very sick and weak, it was etherized and blood drawn from the heart. The autopsy showed a few punctate hemorrhages in the kidneys and a few areas of congestion in the colon. The intestinal contents were bile-stained. The blood was defibrinated and kept on ice in a sterilized flask for 1 day, when the clear serum which had separated from the corpuscles was drawn off with a sterile pipette and diluted with physiological salt solution. A portion of the mixture was now filtered through a Chamberland cylinder "F."

The portion of the diluted serum which was not filtered was preserved for 2 days on ice, when it was filtered through a No. 7 Berkefeld laboratory cylinder, the conditions of filtration being the same as on the previous day when the Chamberland cylinder was used. In both of these filtrations, as well as in all others, every precaution was,

of course, taken to prevent outside contamination, all flasks and filters being thoroughly sterilized before they were used. The culture in bouillon from the unfiltered serum of hog 704 was densely clouded 24 hours after being placed in the incubator. Upon plating from this flask a pure culture of a large organism resembling *B. subtilis* was found. *B. cholerae suis* could not be detected.

The cultures made from the Chamberland and Berkefeld filtrates remained in the thermostat for 3 weeks without showing any signs whatever of bacterial growth.

Rabbit 739, which was inoculated with 10 c. c. of the Berkefeld filtrate, died 33 days after inoculation. The autopsy disclosed no lesions whatever with the exception of echinococcus cysts in the liver. The echinococcus infection was no doubt the cause of death.

Rabbit 740, inoculated with the unfiltered serum, and rabbit 737, and guinea pig 3620, which were both inoculated with the Chamberland "F" filtrate, were all alive and well six months after inoculation.

Hog 749, inoculated with diluted, unfiltered serum, died 30 days after inoculation. There was no local inflammation at the point of inoculation. The inguinal glands were red and somewhat enlarged; spleen engorged with blood, slightly enlarged; kidneys congested, a few punctate ecchymoses in the cortical portion. In the small intestine and in the cecum and colon a number of small erosions were found. The surface of these erosions was covered with a yellowish necrotic material which was easily removed. *B. cholerae suis* was obtained from the liver and spleen.

Hog 748, inoculated with Berkefeld filtrate, died 19 days after inoculation. There was no inflammation at the point of inoculation. Inguinal glands enlarged and dark red; lungs showed two small areas of red hepatization and a few small ecchymoses on the surface. This animal was otherwise normal with the exception of the kidneys, which had undergone a cystic degeneration, the parenchyma of these organs being almost wholly destroyed. Cultures taken from the liver and heart blood failed to reveal the presence of *B. cholerae suis*.

Hog 747, inoculated with Chamberland filtrate, died 27 days after inoculation. There was no inflammation at the point of injection, but the areolar tissue in that region was stained a faint yellow color. The right inguinal gland was considerably enlarged and quite red. The gland on the left side was much smaller and not so red. The liver exhibited on its surface a number of minute points of coagulation necrosis; spleen somewhat enlarged and dark; both kidneys presented a number of punctate hemorrhages on their surfaces; in the neighborhood of the ileocecal valve in the cecum and colon there were a number of comparatively recent ulcers. No cultures were taken from this animal because of postmortem changes which were far advanced when the autopsy was made.

TABLE 15.

No. of hog.	Material injected.	Inoculation.		Autopsy.	Remarks.
		Date.	Result.		
749	10 c. c. diluted unfiltered serum hog 704.	1903. May 27..	1903. Died June 26..	Hemorrhages.....	<i>B. cholerae suis</i> present.
748	11 c. c. diluted filtered serum hog 704.	do....	Died June 15..	do.....	Micrococcus from liver. <i>B. cholerae suis</i> not present.
747	do.....	do....	Died June 23..	Hemorrhages and ulcers.	No cultures taken.

Each of these hogs was kept in a separate pen.

In this experiment the features of especial interest are: (1) The absence of *B. cholerae suis* from the serum of hog 704, both before and after filtration, as determined by cultures and by the inoculation of small animals; (2) the production of fairly well-defined lesions of hog cholera in at least two of the hogs inoculated with this serum; (3) the failure of rabbits and guinea pigs to succumb to doses of the serum which proved fatal to hogs; (4) the presence of *B. cholerae suis* in cultures from hog 749, although a culture test and the inoculation of a rabbit both indicated the absence of this organism from the serum with which the hog was inoculated.

FILTRATION EXPERIMENT No. 2.

Hog 751 was inoculated with 5 c. c. of defibrinated blood from hog 703. Hog 703 was carrying the herd I disease and was quite sick at the time blood was drawn. Hog 751 showed decided symptoms of illness 15 days after inoculation and became gradually worse. Eighteen days after inoculation the animal was etherized and blood was drawn and defibrinated. The lesions found at autopsy were not marked, and did not seem sufficiently grave to account for the severe illness of the animal at the time it was killed. The spleen was enlarged and soft. The mucosa of the cecum and colon was congested and exhibited a number of small erosions, and the intestinal contents were bile stained. The other organs were normal in appearance.

The defibrinated blood, which was collected in a sterile bottle, was kept on ice for 2 days, when the clear serum that had separated from the red cells was withdrawn with a sterile pipette, and diluted with sterile physiological salt solution. After reserving a portion of the diluted unfiltered serum the remainder was divided into three parts and filtered as follows: One portion was filtered through a sterile Berkefeld laboratory cylinder No. 7 (the filtrate was designated "751 Bd"); a second portion of the diluted serum was passed through a sterile Chamberland cylinder marked "F" (filtrate designated "751 Ch. F"); the third portion was passed through a sterile Chamberland cylinder marked "B" (filtrate designated "751 Ch. B"). In the case of the Chamberland filtrations the cylinders were arranged in the manner shown in plate 2. From each of these three filtrates 25 c. c. was removed by means of a sterile pipette and added to a flask containing 400 c. c. of neutral beef broth. At the same time, 2 c. c. of the undiluted serum of hog 751 was added to 400 c. c. of sterile beef broth. All four of these flasks were placed together in a thermostat at 37.5° C.

The flasks inoculated with the unfiltered serum and with the Chamberland "F" filtrates became clouded, while those inoculated with the Berkefeld and Chamberland "B" filtrates remained clear after being in the incubator for 9 days. An examination of the culture from the

unfiltered serum showed it to contain a micrococcus in pure culture, while the flask inoculated with the Chamberland "F" filtrate contained a pure culture of a large bacillus which resembled *B. subtilis*. No attempt was made to identify these organisms, the examination being carried only so far as was necessary to ascertain that *B. cholerae suis* was absent. The presence of the large bacillus in the culture from the Chamberland "F" filtrate, in view of its absence from the unfiltered serum, can only be accounted for by accidental contamination from the air, for which there was abundant opportunity during the removal of the 25 c. c. used for the culture.

Hog 766 was inoculated with defibrinated blood of 751 immediately after it was drawn, and placed alone in a pen; was first noticed to be sick in 11 days, and died in 14 days. The autopsy revealed numerous small hemorrhages and some edema in the lungs. The liver and spleen were enlarged and congested; the kidneys hemorrhagic. There was considerable pigmentation, and there were also numerous small hemorrhages in the intestine; a few recently formed ulcers in the colon.

Hog 788 was inoculated with diluted, not filtered, serum and placed alone in a pen; was first noticed to be quite sick in 9 days, and remained so for 18 days, when improvement began. This improvement continued for 30 days, when the animal had apparently completely recovered, so it was placed in the exposure pen to test its immunity. The hog was killed after remaining in the exposure pen for about 7 months, where other hogs were dying of hog cholera. It was in an unthrifty condition, but no lesions of hog cholera could be found.

Hog 783 was inoculated with Berkefeld filtrate; was first noticed to be sick in 8 days. This hog was in a moribund condition when killed 15 days after inoculation. The legs and ears were reddened and there were many red areas over the ventral surface of the body. There was no lesion at the point of inoculation. The lymph glands were edematous and there were several areas of red collapse in the lungs. The kidneys were hemorrhagic; the intestines appeared normal, although their contents were bile-stained.

Hog 789, inoculated with Berkefeld filtrate, showed symptoms of illness in 8 days and died in 11 days. There was extreme reddening of the ventral surface of the body and of the head and ears; the lungs were sprinkled throughout with ecchymoses varying from 3 to 15 mm. in diameter; spleen enlarged and engorged with blood; kidneys presented a few punctiform hemorrhages; some ecchymoses and ulcers in the mucous membrane of the intestines. Hogs 783 and 789 were placed in a pen by themselves.

Hog 790, inoculated with Chamberland "F" filtrate, was slightly sick in 11 days and became gradually worse and died 28 days after inoculation. The ventral surface of the body was extensively reddened; the lymphatic glands were enlarged and red; the kidneys hemorrhagic, and cecum and colon extensively ulcerated.

Hog 791, a mate to hog 790, was injected on the same date with same lot of serum; was seen to be sick in 7 days, became rapidly worse, and died in 12 days. There was extreme reddening of the ventral surface of the body and of the ears; no lesion at the seat of injection, but the adjacent lymph glands were enlarged and hemorrhagic; the lungs were sprinkled throughout with ecchymoses; the spleen enlarged and congested; the kidneys presented punctiform hemorrhages in the cortices; both large and small intestines were flecked with hemorrhages of varying size, but no ulcers could be found. Hogs 790 and 791 were placed in the same pen.

Hog 784, inoculated with Chamberland "B" filtrate, became sick 6 days after inoculation and died in 12 days. The autopsy revealed lesions which belong exclusively to the hemorrhagic type of disease. There was extensive reddening of the skin, reddening and swelling of the lymphatic glands, but no inflammation at the seat of injection. Ecchymoses of varying size and number were present in the lungs, spleen, liver, kidneys, stomach, and large and small intestines.

Hog 786, inoculated at the same time and in the same manner as hog 784, became slightly sick 8 days after inoculation, and being hopelessly sick on the fifteenth day, was killed. The lesions found belonged to the chronic intestinal type, and therefore differed quite markedly from those met with in its mate, hog 784. The only explanation of the wide difference in the lesions found in these 2 hogs seems to lie in a variation in resisting power possessed by the 2 animals. Hogs 784 and 786 had been placed in a pen by themselves.

TABLE 16.

No. of hog.	Material injected.	Inoculation.		Autopsy.	Remarks.
		Date.	Result.		
		1903.	1903.		
766	5 c. c. defibrinated blood hog 751	June 15	Sick June 26; died June 29	Hemorrhages.	No cultures taken.
788	20 c. c. unfiltered serum hog 751	June 18	Very sick; recovered	Hemorrhages.	Immune when exposed subsequently.
789	20 c. c. serum 751 "Bd."	do	Sick June 26; killed July 3	do	In dying condition when killed.
790	do	do	Sick June 26; died June 29	do	No cultures taken.
791	20 c. c. serum 751 "Ch. F."	do	Sick June 29; died July 16	Hemorrhages and ulcers.	Do.
792	do	do	Sick June 25; died June 30	do	Do.
784	20 c. c. serum 751 "Ch. B."	do	Sick June 24; died June 30	do	Do.
786	do	do	Sick June 26; killed July 3	Ulcers.	In dying condition when killed.

The results of Filtration Experiment No. 2 are even more striking than those obtained in Filtration Experiment No. 1. Flasks inoculated with 25 c. c. of the diluted serum—5 c. c. more than was employed for inoculating a hog—failed to reveal the presence of *B. cholerae suis* in either the filtered or unfiltered serum. It seems extremely improbable that *B. cholerae suis* could have been present in all three of the filtrates and also in the unfiltered serum in sufficient numbers to produce acute hog cholera by subcutaneous injection and yet have failed to grow in the cultures from any of them. The possibility of accidental infection from without or from the pens or syringes also seems to be highly improbable in view of the uniformity of the results, and the great rarity of such accidental infection in other experiments where hogs were inoculated with substances other than the blood or tissues of hogs sick of hog cholera.

Notwithstanding the unlikelihood of the disease in these hogs having been caused by any other agent than the injected serum, plans were made for another set of experiments which would eliminate still further the chances of error mentioned above.

FILTRATION EXPERIMENT No. 3.

Hog 797, the animal which furnished the blood for this experiment, was inoculated subcutaneously with 5 c. c. of blood from hog 792, which was carrying herd I disease. Hog 797 became very sick 11 days after inoculation, and was etherized and blood drawn from the heart and defibrinated. The autopsy on hog 797 disclosed hemorrhagic kidneys and a number of recent ulcerations in the cecum and colon.

The blood obtained from hog 797 was kept on ice for 3 days. The serum was then withdrawn with a sterile pipette and mixed with sterilized physiological salt solution. Different portions of this diluted

serum were then filtered through three sterilized filters—a Berkefeld laboratory cylinder No. 7 (filtrate designated “797 Bd.”), a Chamberland cylinder “F” (filtrate designated “797 Ch. F.”), a Chamberland cylinder “B” (filtrate designated “797 Ch. B.”).

The Chamberland cylinders were so arranged that the filtration should take place from within outward, as in plate 2; the Berkefeld cylinder was arranged in the usual manner, shown in plate 1. After completing the several filtrations, cultures were made as follows:

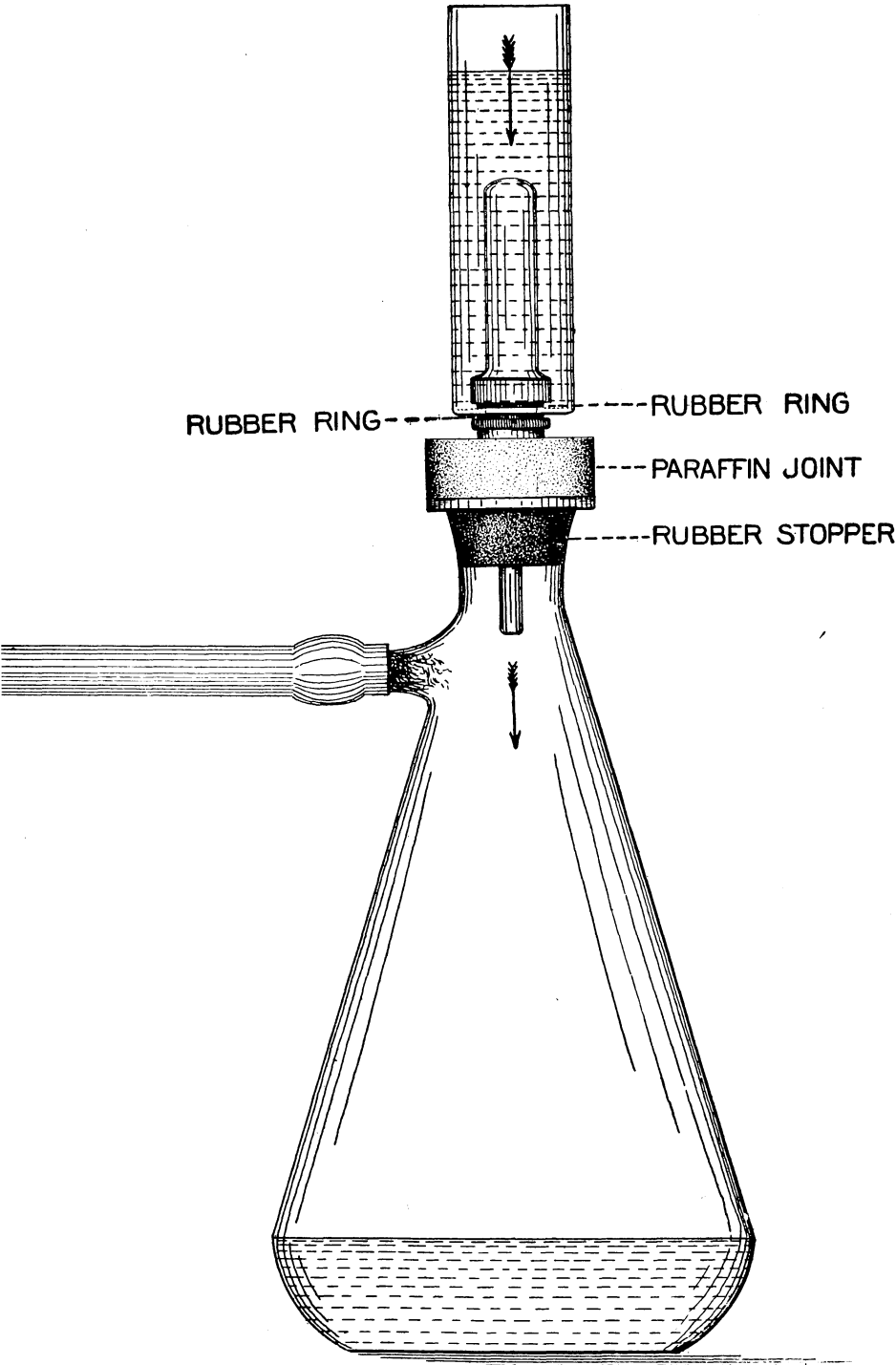
To flasks containing about 400 c. c. of sterile neutral bouillon 25 c. c. of each of the filtrates was added, and to a similar flask 4 c. c. of the original undiluted serum. All cultures were placed immediately in the incubator at 37.5° C. Three days after these cultures were prepared, the one made from the undiluted and unfiltered serum showed a dense clouding, and the cultures from the Chamberland “F” and “B” filtrates were both slightly cloudy. The culture from the Berkefeld filtrate remained clear. In order to ascertain whether *B. cholerae suis* was present in either of these cultures, guinea pigs were inoculated with $\frac{1}{2}$ c. c. of each. The guinea pig inoculated with the culture from the unfiltered and undiluted serum remained well. The same is true of the guinea pig inoculated from the Chamberland “B” culture. The one inoculated with the Chamberland “F” culture died 25 days after inoculation. A careful bacteriological examination, however, failed to reveal the presence of *B. cholerae suis*, but indicated that death was due to infection by an entirely different organism.

Immediately after defibrination the blood of hog 797 was injected subcutaneously into hogs 819 and 820, 5 c. c. into each. The inoculations with the diluted serum of hog 797 were made after the serum had been in the refrigerator overnight.

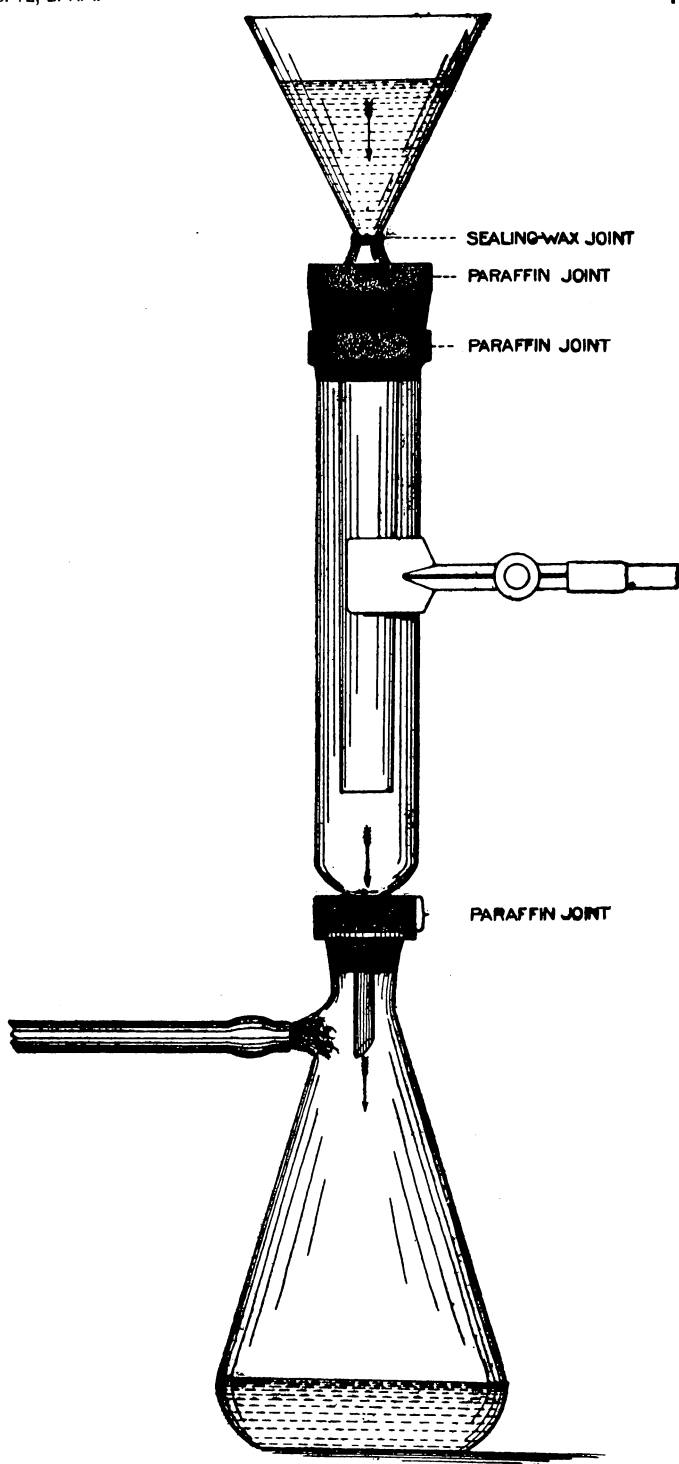
The numbers of the hogs concerned and their arrangement in the pens are as follows:

Hog 819.	Received 5 c. c. defibrinated blood of hog 797	} Placed in same pen.
820.	Do	
837.	Received 22 c. c., diluted, unfiltered serum of hog 797.....	} Do.
827.	Uninoculated pen check	
832.	Received 22 c. c. serum hog 797, filtered through Berkefeld	} Do.
834.	Do	
830.	Uninoculated pen check	} Do.
828.	Received 22 c. c. serum hog 797, filtered through Chamberland “F”	
829.	Do	} Do.
836.	Uninoculated pen check	
824.	Received 22 c. c. serum hog 797, filtered through Chamberland “B”	} Do.
833.	Do	
838.	Uninoculated pen check	} Do.
825.	Received in same manner, 22 c. c. sterile bouillon	
826.	Do	} Do.
831.	Uninoculated pen check	

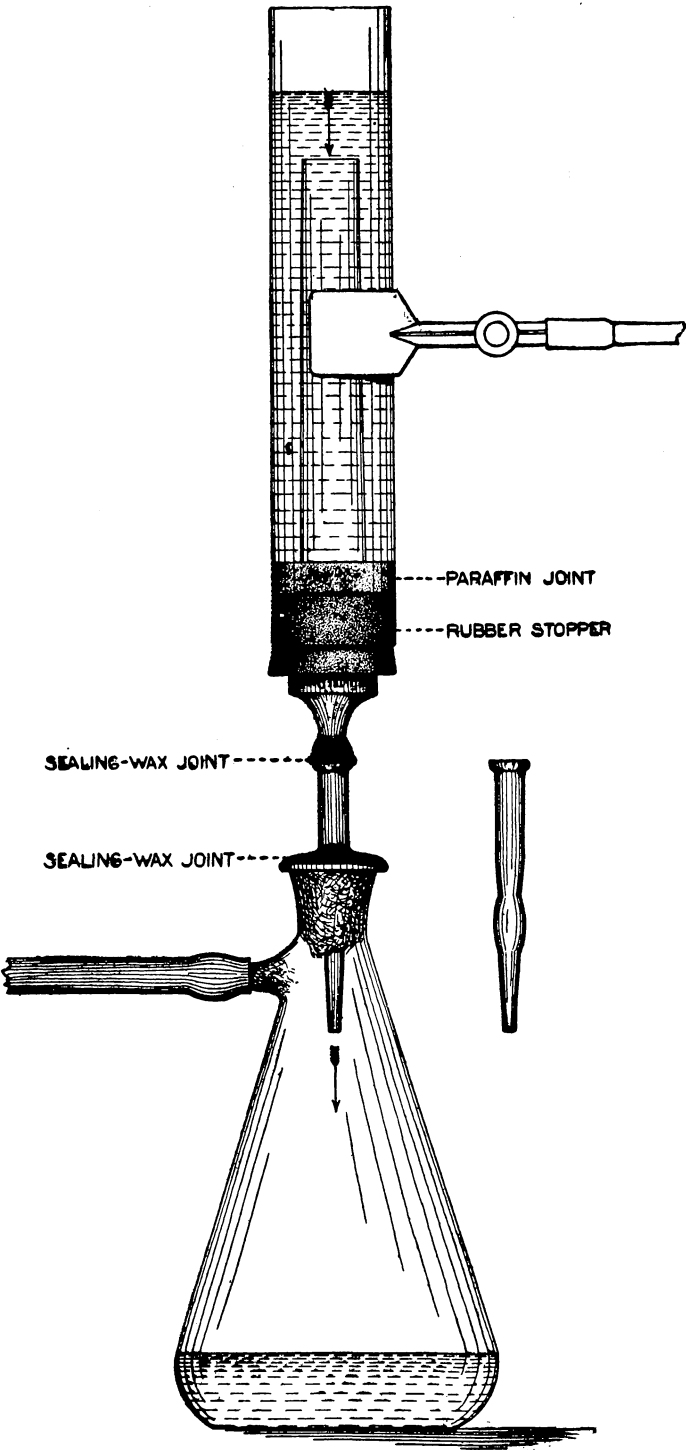
Hog 819 was inoculated with 5 c. c. of defibrinated blood of hog 797; was desperately sick in 8 days and was etherized in 9 days in order that blood might be drawn



BERKEFELD FILTER.



CHAMBERLAND FILTER.



CHAMBERLAND FILTER.

for other experiments. The autopsy revealed minute hemorrhages in the lungs, enlarged spleen, congested kidneys; intestinal contents were bile-stained, but no ulcers could be found.

Hog 820 was inoculated at the same time and with the same material as hog 819. This hog became desperately ill and was etherized 9 days after inoculation in order that blood might be obtained for other experimental work. The autopsy was quite similar to that of hog 819, the only noticeable difference being that in the case of hog 820 the intestines were congested and some hemorrhages were found on the mucous coat.

Hog 837 was inoculated with diluted, not filtered, serum of hog 797; showed no symptoms of illness for 7 days, when it appeared to be very sick, and the symptoms increased in severity till death, 11 days after inoculation. There was no inflammation at the point of inoculation. The inguinal glands were slightly reddened; there were a few small hemorrhagic areas in the lungs. The spleen was enlarged and dark; a few punctiform hemorrhages were seen in the kidneys. The intestinal contents were fluid and faintly tinged with blood. The mucous membrane of the cecum and colon presented a few small ecchymoses, and in the cecum some small ulcers were found, evidently of recent formation. From the spleen of this animal a pure culture of a microorganism resembling *B. cholerae suis* in all of its cultural characters except in its action upon saccharose was obtained. This sugar underwent fermentation with the production of gas.

Hog 827 was not inoculated, but placed as a check in the pen with hog 837; showed no signs of illness for 14 days, when symptoms similar to those seen in 837 developed, and the hog died 4 days later. The subcutaneous areolar tissue over the abdomen and thorax was sprinkled with small ecchymoses. The inguinal, lumbar, mesenteric, and mediastinal glands were enlarged and reddened. Numerous petechiae on the surface of the heart muscle. The spleen was somewhat enlarged and quite dark; kidneys were dotted with numerous punctiform hemorrhages; the peritoneum contained a number of discrete hemorrhages. A few small hemorrhages were found on the serous surface of the stomach, and also on the serous and mucous surfaces of the small intestines, cecum, and colon. No ulcers could be found. Cultures from the spleen revealed the presence of *B. cholerae suis*.

Hog 832 was inoculated with the Berkefeld filtrate from serum of hog 797; was quite sick in 7 days and died in 12 days. There was no lesion at the seat of injection. The lungs were sprinkled throughout with minute hemorrhages. The spleen was greatly enlarged and engorged with blood; the kidneys thickly sprinkled with punctiform hemorrhages. The small intestine exhibited numerous circumscribed hemorrhages in the mucosa. The mucosa of the cecum and colon contained many small ulcers and the intestinal contents were blood-stained. No cultures were taken.

Hog 834 was injected with the same material at the same time as hog 832; was quite sick 7 days after inoculation, became rapidly worse, and died on the twelfth day. There were no lesions at the seat of injection. The inguinal glands were enlarged and reddened; there were a few small ecchymoses on the surfaces of the lungs; the spleen was enlarged and congested; the kidneys were also congested and in addition were dotted with minute sharply defined hemorrhagic points. A few superficial erosions were found on the mucous surface of the cecum and colon. Cultures from the spleen revealed the presence of *B. cholerae suis*.

Hog 830 was not inoculated, but served as a pen check on hogs 832 and 834; seemed to be slightly sick in 11 days; was desperately sick in 15 days and was killed. There were no lesions at the seat of inoculation. The inguinal, mediastinal, and mesenteric glands were all enlarged and very red. There were diffuse hemorrhages in the epicardium and a large number of minute, sharply defined hemorrhages on the surfaces of the lungs. The spleen was very much enlarged; the kidneys showed the characteristic punctiform hemorrhages in their cortices. The peritoneum was dotted with numerous small ecchymoses, as were the serous surfaces of the stomach, cecum, and colon.

Hog 828 was inoculated with Chamberland "F" filtrate from serum of hog 797; first sick in 7 days and was found dead in 12 days. The inguinal glands were somewhat reddened. On the surfaces of the lungs a few small hemorrhagic points could be seen. The spleen was enlarged, cecum and colon congested, and on the mucous surface certain small areas were covered with a tenacious yellow exudate which, upon being removed, revealed a rather superficial erosion of the mucosa.

Hog 829 was treated in the same manner as hog 828; first signs of sickness in 7 days; was found dead in 12 days. The autopsy was almost identical with that of hog 828. Cultures from the spleen revealed *B. cholerae suis*.

Hog 836 was not inoculated, but served as a pen check on hogs 828 and 829. The first symptoms of illness were noted in 12 days and the animal died in 17 days. A few small hemorrhages were found on the auricular appendages of the heart. The

TABLE 17.

No. of hog.	Material injected.	Inoculation.		Autopsy.	Remarks.
		Date.	Result.		
819	5 c. c. defibrinated blood hog 797	1903. July 14	1903. Sick July 22; killed July 23	Hemorrhages	No cultures taken.
820	do	do	do	do	Do.
837	22 c. c. unfiltered serum hog 797	July 20	Sick July 27; died July 31	do	Atypical <i>B. cholerae suis</i> from spleen.
827	Not inoculated; pen check on hog 837	Exp'd July 20	Sick Aug. 3; died Aug. 7	do	No cultures taken.
834	22 c. c. serum hog 797 "Bd."	July 20	Sick July 27; died Aug. 1	do	Do.
832	do	do	do	do	No cultures taken.
830	Not inoculated; pen check on hogs 832 and 834	Exp'd July 20	Sick July 31; killed Aug. 3	do	<i>B. cholerae suis</i> from spleen.
828	22 c. c. serum hog 797 "Ch. F."	July 20	Sick July 27; died Aug. 1	Hemorrhages and ulcers	Do.
829	do	do	do	do	No cultures taken.
836	Not inoculated; pen check on hogs 828 and 829	Exp'd July 20	Sick Aug. 1; died Aug. 6	do	<i>B. cholerae suis</i> from spleen.
824	22 c. c. serum hog 797 "Ch. B."	July 20	Sick July 27; died Aug. 1	do	No cultures taken.
833	do	do	Sick July 27; died July 31	do	Atypical <i>B. cholerae suis</i> from spleen.
838	Not inoculated; pen check on hogs 824 and 833	Exp'd July 20	Sick Aug. 3; died Aug. 11	do	<i>B. cholerae suis</i> from spleen.
825	22 c. c. sterile bouillon	July 20	Remained well	do	do
826	do	do	do	do	do
831	Not inoculated; pen check on hogs 825 and 826	Exp'd July 20	do	do	do

spleen was enlarged and quite dark; the kidneys were very much congested and exhibited some punctiform hemorrhages on their surfaces. Some small petechiae on the serous surface of the stomach. The small intestine presented on its serous surface a large number of small, rather sharply defined ecchymoses. The serous surfaces of the cecum and colon were less affected than the same portion of the small intestine. The mucous coat of the latter presented many small erosions, the centers of which were of a reddish color. The contents of the cecum were semifluid and contained some blood.

Hog 824 was inoculated with Chamberland "B" filtrate from serum of hog 797; was first noticed to be sick in 7 days; became rapidly worse and died 12 days after inoculation. The entire ventral surface of the body was reddened. The inguinal, lumbar, and mesenteric glands were enlarged and red. There were numerous scattered hemorrhagic areas on the surfaces of the lungs and the cephalic lobe of the left lung was hepatized. The spleen was enlarged and dark with irregular diffuse hemorrhagic areas on its surface. The kidneys were thickly dotted with punctiform hemorrhages. There were numerous small petechiae on the serous and mucous surfaces of both the large and small intestines. The mucous membrane of the cecum and colon contained a number of small ulcers.

Hog 833 was inoculated at the same time with the same material as hog 824; was quite sick in 7 days and became rapidly worse, dying 12 days after inoculation. There were no lesions found at the seat of inoculation. The inguinal glands were enlarged and red, and there were many sharply defined hemorrhages on the surfaces of the lungs; the spleen was somewhat enlarged; the kidneys congested and dotted with numerous punctiform hemorrhages. Some hemorrhages were found on the surfaces of the stomach and also on the serous and mucous surfaces of the intestines. The intestinal contents were blood-stained.

Hog 838 was not inoculated, but placed as check in the pen with hogs 824 and 833. The animal remained well for 14 days, when it became suddenly ill and died 22 days after exposure. The lungs contained, besides several areas of red collapse, numerous small hemorrhages. Both of the auricles of the heart were

sprinkled with petechiæ. The spleen was enlarged, kidneys hemorrhagic, and innumerable minute hemorrhages were found on the mucosa of the small intestine; a number of ulcers were present in the mucosa of the cecum and colon. Cultures from the spleen contained pure growths of *B. cholerae suis*.

Hogs 825, 826, and 831: Two of these hogs, 825 and 826, were injected with sterile bouillon, the other, 831, serving as a check. All 3 remained perfectly well for 40 days. Hogs 825 and 826 were then injected with blood from a hog sick with herd II disease, hog 831 being again used as a check. All 3 died, thus showing that their failure to contract disease when used in "Filtration Experiment No. 3" was not due to natural immunity.

It is evident that the results obtained in Filtration Experiment No. 3, substantiate completely the work described under Filtration Experiments Nos. 1 and 2. *B. cholerae suis* could not be found in any of the material used for injection either before or after filtration, and yet the hogs inoculated with this material sickened promptly and the majority of them died, typical lesions of hog cholera being found in every instance. Filtration Experiment No. 3, was of greater value than the two preceding experiments on account of the check animals which were used in connection with the different inoculations. A glance at the accompanying table will show that in every instance the injected animals became sick from 4 to 7 days sooner than the accompanying pen checks, thus indicating in a direct way that the infection arose from the injected material and not from the pens in which the animals were kept. Hogs 825 and 826, which were handled in the same manner as the remainder of the animals in this experiment, but which were injected with sterile bouillon instead of with the filtered diseased serum, remained perfectly well, thus furnishing proof that the disease in the hogs which sickened could not be attributed to an infection through careless handling or through the introduction of diseased material into the pens subsequent to inoculation. We would call especial attention to the fact that the subcutaneous injections of filtered blood not only caused the death of the injected animals, with typical lesions of hog cholera, but that the disease thus produced was contagious for susceptible animals placed in the pen with those which were injected.

FILTRATION EXPERIMENT NO. 4.

The blood used in this experiment was obtained from hog 1113, inoculated with 7 c. c. of defibrinated blood of hog 1107 carrying herd I disease. Hog 1113 became very sick on the sixth day after inoculation and was then etherized to obtain blood for the experiment which follows. The autopsy on hog 1113 revealed several small areas of congestion in the lungs; numerous small gray areas and a few hemorrhagic spots in the liver; a few small hemorrhagic areas in the spleen; kidneys congested; fundus of stomach congested; mucosa of ileum covered with a thin, yellow exudate; numerous small, hemorrhagic points and small erosions in the mucosa of cecum and colon. Cultures from heart blood showed *B. cholerae suis*.

The heart blood of hog 1113, drawn by means of a sterile canula, was defibrinated, filtered through absorbent cotton, and allowed to stand in the refrigerator for 2 days. Of the clear, supernatant serum, 50 c. c. was drawn off and added to 500 c. c. of sterile normal salt solution so as to dilute the serum in the proportion of 1:10. After thorough mixing, 50 c. c. of this diluted serum was drawn off for the inoculation of experiment animals, and 3 c. c. of undiluted serum was added to each of the two flasks containing 400 c. c. each of neutral beef broth. To the diluted serum was next added 3 c. c. of a 24-hour bouillon culture of *B. pyocyaneus*, and the entire mixture was then passed through a Chamberland "F" filter. Cultures were made from the filtrate by drawing off 50 c. c. with a sterile pipette and adding 25 c. c. to each of two flasks of neutral beef broth (each containing 400 c. c.).

The cultures from the diluted serum before filtration showed *B. cholerae suis*. Of the two flasks inoculated with the diluted filtered serum, one showed clouding after 42 hours in the incubator and agar plates revealed a pure growth of a large, heavy, sluggishly motile bacillus, corresponding to *B. subtilis*; the other flask remained sterile. It would seem safe to conclude, therefore, that the filtrate used in this experiment was entirely free from *B. cholerae suis*.

In carrying out the experiment, 2 hogs, 1137 and 1138, were inoculated with 20 c. c. each of the diluted filtered serum and placed together in one pen. Another hog, 1139, was inoculated with 21 c. c. of the diluted unfiltered serum and placed in a separate pen. Besides these hogs, a series of small animals, including rabbits, guinea pigs, white rats, white mice, and gray mice, were inoculated with the filtered and unfiltered serum and placed in separate cages.

The following is a list of the animals inoculated, with brief notes and autopsy records in each case:

Hog 1139 was injected subcutaneously with 21 c. c. of diluted unfiltered serum of hog 1113; was placed alone in a pen. This hog was first noticed sick after 5 days, became rapidly worse, and died 16 days after injection. Autopsy showed reddening of skin about nostrils and over ventral surface of body; lungs congested and edematous; numerous petechiæ and several large patches of extravasation in the heart muscle; liver rather pale, but many of the individual lobules intensely reddened; spleen enlarged and thickly studded with small, punctate hemorrhages; numerous small hemorrhages scattered over serous surface of stomach and small intestine; mucosa over fundus of stomach reddened; mucosa of small intestine marked with hemorrhagic lines and points; mucosa of cecum showed a few hemorrhagic points, which were more numerous throughout the colon; mucosa of rectum also sprinkled with hemorrhagic points. No cultures were made from this animal.

Hog 1137 was injected subcutaneously with 20 c. c. of the diluted filtered serum of hog 1113. The animal was slightly sick from the ninth to twelfth day after inoculation, but apparently recovered; was in fairly good condition when placed in exposure pen 34 days after inoculation. Died after an exposure of 88 days. Autopsy showed inguinal, mediastinal, and mesenteric glands enlarged and dark; liver pale, and in places the lobules showed as hemorrhagic points; kidneys dark, with the appearance of chronic interstitial nephritis; mucosa of stomach showed an area of congestion toward pyloric end; cecum showed grayish indurations, having the appearance of old, healed ulcers. Cultures from heart blood, liver, and spleen showed *B. cholerae suis*.

Hog 1138 was injected subcutaneously with 20 c. c. of the diluted filtered serum of hog 1113. The animal was slightly sick from the ninth to the twelfth day after inoculation, but apparently recovered; was in fairly good condition when placed

in exposure pen 35 days after inoculation. The animal became thin and runty, and was killed after an exposure of 101 days. The autopsy revealed numerous areas of congestion in the lungs; liver of a pale, yellow-gray color, and very soft and necrotic; a number of old, healed ulcers in the cecum and colon. No cultures were made from this animal.

SMALL ANIMALS INOCULATED SUBCUTANEOUSLY WITH UNFILTERED SERUM OF HOG 1113.

Rabbit 995 received 6 c. c. and died 10 days after inoculation. Autopsy showed a small patch of necrosis in each groin at site of inoculation; liver much enlarged, pale in color, soft, and friable; spleen enlarged and dark; Peyer's patches reddened. *B. cholerae suis* present in cultures from heart blood, liver, and spleen.

Guinea pig 4528 received 3 c. c. and died 20 days after inoculation. Autopsy showed considerable extravasation in subcutaneous tissues at site of inoculation; axillary, inguinal, and mesenteric glands red and swollen; lungs slightly congested; liver dark and mottled, with small, scattered areas of necrosis; spleen much enlarged and dark, with small, necrotic areas; kidneys pale and mottled; Peyer's patches swollen. Cultures from heart blood, liver, and spleen showed *B. cholerae suis*.

White rat received $1\frac{1}{2}$ c. c. and died 7 days after inoculation. Autopsy showed inguinal glands on left side reddened; lungs much congested; liver somewhat enlarged, pale, and mottled; spleen enlarged and dark; kidneys somewhat congested; pyloric end of stomach and small intestine hyperemic. Cultures from lungs showed *B. cholerae suis*.

White mouse received $\frac{1}{2}$ c. c. and died 21 days after inoculation. Autopsy showed considerable congestion of the lungs; liver dark and covered with a tenacious mucopurulent exudate; spleen greatly enlarged and dark, with several small areas of necrosis. Cultures from heart blood, liver, and spleen showed *B. cholerae suis*.

SMALL ANIMALS INOCULATED SUBCUTANEOUSLY WITH FILTERED SERUM OF HOG 1113.

Guinea pig 4529 received 3 c. c. and died 6 days after inoculation. Autopsy showed inguinal glands on left side slightly enlarged and hyperemic; slight congestion of the lungs; a few small, pin-point gray areas in liver; spleen somewhat darker than normal; kidneys dark and somewhat mottled, especially the right, which showed considerable congestion. Cultures from heart blood and organs failed to show *B. cholerae suis*.

Rabbit 1000 received 6 c. c. and remained well.

White rat received $1\frac{1}{2}$ c. c. and remained well.

Gray rat received $1\frac{1}{2}$ c. c. and remained well.

Gray mouse received $\frac{1}{2}$ c. c. and remained well.

TABLE 18.

No. of hog.	Material injected.	Inoculation.		Autopsy.
		Date.	Result.	
1139	21 c. c. diluted unfiltered serum hog 1113.	1904. Mar. 5	1904. Sick Mar. 10; died Mar. 21.	Hemorrhages.
a 1137	20 c. c. diluted filtered serum hog 1113.	do....	Sick Mar. 14 to 17; recovered.	
b 1138	do.....	do....	do.....	

^a Hog 1137 was placed in the exposure pen on Apr. 8 and died July 5, 1904, after an exposure of 88 days. The autopsy revealed some of the lesions of hog cholera, chiefly chronic in character, and *B. cholerae suis* was present in cultures from the heart blood.

^b Hog 1138 was placed in the exposure pen on Apr. 9 and became thin and runty, and was killed July 19, 1904, after an exposure of 101 days. The autopsy revealed old chronic ulcers in the cecum and colon. No cultures were made from this animal.

In this experiment the 2 hogs inoculated with filtered serum were both slightly sick as a result of the inoculation, but apparently recovered and were later placed in the exposure pen. One of these animals, hog 1137, died in the exposure pen after 88 days with interstitial lesions and healed ulcers in the cecum; cultures showed *B. cholerae suis*; the other, hog 1138, became thin and runty, and was

killed after being in the exposure pen for 101 days, and showed lesions similar to those seen in hog 1137. It would seem probable that these animals were both rendered immune as a result of the injection of filtered serum, but that they had not fully recovered when placed in the exposure pen; and that the death of one and poor condition of the other was caused by malnutrition and unfavorable surroundings rather than by the specific disease to which they were exposed.

The hog inoculated with unfiltered serum died quite promptly as a result of the inoculation and showed hemorrhagic lesions. Of the small animals, all of those inoculated with unfiltered serum died as a result of the inoculation, and *B. cholerae suis* was recovered from the heart blood and internal organs, while of those inoculated with filtered serum none died as a result of the inoculation.

FILTRATION EXPERIMENT No. 5.

The blood used in this experiment was obtained from hog 1159, one of a series of animals carrying herd I disease.

The heart blood of hog 1159 was drawn and defibrinated in the usual manner and then allowed to remain in the ice box for 48 hours. Fifty cubic centimeters of the clear serum was then drawn off with a sterile pipette and mixed with 500 c. c. of neutral beef broth. A portion of this diluted serum was used for the inoculation of a hog and for cultures. The latter showed after incubation a pure growth of *B. cholerae suis*.

Three large loops from the water of condensation of an active agar culture of *B. pyocyaneus* were next added to the diluted serum and the whole mixture passed through a Chamberland "F" filter under a vacuum of 23 inches of mercury, the time of filtration being 28 minutes.

A portion of the clear filtrate was drawn off with a sterile pipette and used for the inoculation of hogs. The remainder of the filtrate, about 375 c. c., was placed in the incubator and at the end of 48 hours showed a pure growth of a large heavy bacillus resembling *B. subtilis*—in all probability a contamination from the air.

Hog 1176 was injected subcutaneously with 55 c. c. of diluted unfiltered serum of hog 1159; showed first symptoms of sickness on eighth day, became rapidly worse, and died 15 days after inoculation. Autopsy showed inguinal glands and lymphatics generally swollen and hyperemic; lungs edematous and sprinkled with small hemorrhagic patches; a few small hemorrhages over the auricles; liver congested, with thickening of connective tissue elements; spleen greatly enlarged and dark; kidneys swollen and thickly studded with small punctate hemorrhages; mucosa of stomach showed numerous small hemorrhagic points and over fundus was markedly hyperemic; mucosa of small intestine showed numerous punctiform hemorrhages and ulceration along the folds; mucosa of cecum and colon intensely pigmented with numerous circular or oval, flat ulcers, and numerous hemorrhagic patches. No cultures were taken from this animal.

Hog 1177 was injected subcutaneously with 55 c. c. of diluted filtered serum from hog 1159 and placed in a pen with hogs 1178 and 1179; showed first symptoms of sickness on tenth day; became rapidly worse and died 14 days after inoculation. Autopsy showed lungs edematous with numerous small hemorrhagic points; peri-

TABLE 19.

No. of hog.	Material injected.	Inoculation.		Autopsy.	Remarks.
		Date.	Result.		
1176	55 c. c. diluted, unfiltered serum hog 1159.....	1904. Apr. 19	1904. Sick Apr. 27; died May 4.....	Hemorrhages and ulcers...	No cultures taken.
1177	55 c. c. diluted, filtered serum hog 1159.....	do	Sick Apr. 29; died May 3.....	do	No <i>B. cholerae suis</i> present.
^a 1179	do	do	Sick Apr. 30 to May 15; recovered.....		
^b 1178	Not inoculated; exposed to hogs 1177 and 1179.....	(c)	Sick May 4 to 16; recovered.....		

^a Hog 1179 was placed in exposure pen May 23; was alive and in good condition Feb. 6, 1905.

^b Hog 1178 was placed in exposure pen on May 23, and remained well for 6½ months.

^c Exposed April 19.

cardial sac distended with blood-stained serum, and the epicardium and endocardium showed numerous echymoses; spleen greatly enlarged and dark; liver showed thickening of connective tissue and a few lobules were hemorrhagic; kidneys pale with a few punctate hemorrhages; a few small hemorrhagic points on the serous and mucous surfaces of the stomach and small intestine; mucosa of cecum and colon intensely pigmented, and the cecum showed a number of typical "button" ulcers. Cultures from heart blood failed to show *B. cholerae suis*.

Hog 1179 was injected subcutaneously in the same manner as hog 1177; showed first symptoms of sickness on the eleventh day after inoculation, and was quite sick for about a week, but recovered and was placed in exposure pen 34 days after inoculation. Was alive and in good condition 8 months later.

Hog 1178 was placed in the pen with hogs 1177 and 1179 as a check; was slightly sick 15 days later and became worse, but finally recovered and was placed in exposure pen 34 days after first exposure to hogs 1177 and 1179; was kept in exposure pen 6½ months and remained well.

In summarizing the results obtained in this experiment we find that 1176, the hog inoculated with unfiltered serum, and 1177, one of the hogs that received filtered serum, died at the end of 15 and 14 days, respectively, and both showed hemorrhagic lesions and ulcers in the cecum.

Hog 1179, the other animal inoculated with filtered serum, became quite sick, but recovered and was subsequently immune, as shown by an exposure of 8 months in the exposure pen.

Hog 1178, the pen check, exposed to the two filtered-serum animals, did not become sick until 4 days after the inoculated animals, thus indicating that the disease was caused by the filtered serum, and also that it was contagious. This hog, 1178, was subsequently found to be immune when placed in the exposure pen.

In this experiment the filtrate showed clouding after 48 hours in the incubator, but agar plates made from this filtrate, subsequent to clouding, showed a pure growth of a large bacillus resembling *B. subtilis*.

FILTRATION EXPERIMENT NO. 6.

The blood used in this experiment was obtained from hog 1190, which was injected

inside of left thigh with 1 c. c. of defibrinated blood of hog 1166. The latter animal was one of a series of animals carrying the herd I disease. Hog 1190, being quite sick, was etherized and blood was drawn from the heart 7 days after inoculation. The postmortem examination of hog 1190 disclosed extensive hemorrhagic lesions. Two filtrations of the blood of hog 1190 were made as follows:

Filtration A.

The defibrinated blood was kept for 1 day in the refrigerator. The serum was then drawn off and 50 c. c. of it was diluted with 500 c. c. of neutral beef broth. The diluted serum was then inoculated with a culture of *B. prodigiosus* and filtered through a Chamberland "F" cylinder. The filtration took place from within outward, as shown in plate 2. Of this filtrate 25 c. c. was added to a flask of about 400 c. c. of beef broth, 100 c. c. was used for the subcutaneous injection of 2 hogs (50 c. c. into each), and 11 c. c. was injected into a guinea pig. A beef-broth culture was also made from the unfiltered blood.

The inoculation made into beef broth from the unfiltered blood showed growth of *B. cholerae suis*; that from the filtered blood showed no growth of any kind, and the guinea pig inoculated with the filtered blood lived 117 days.

Hogs 1208 and 1209 were each injected subcutaneously with 5 c. c. of the undiluted defibrinated blood of hog 1190 immediately after it was drawn. Hog 1208 began to show symptoms of sickness on the seventh day, became rapidly worse, and was killed on the tenth day in order to obtain blood for other experiments. The autopsy on this hog revealed very slight lesions; in fact, practically none that could be regarded as characteristic of hog cholera. *B. cholerae suis* was obtained from the organs in pure culture. (The blood of this animal was used in connection with Tail-blood Experiment No. 2.)

Hog 1209 was first noticed sick on the seventh day and was found dead on the following day. The autopsy revealed typical lesions of acute hog cholera. The spleen was enlarged and dark and the mucosa of the stomach congested, kidneys dotted with punctiform hemorrhages, and many small ecchymoses were found in the mucosa of small intestine, cecum, and colon. There were numerous small erosions in the mucous membrane of the colon. Agar cultures from heart blood showed pure cultures of *B. cholerae suis*.

Hog 1212 was injected with 47 c. c. of filtered serum from hog 1190 and placed in a pen with hog 1213, which received the same, and with hog 1214, an uninoculated pen check. Hog 1212 remained well and was removed to the exposure pen 29 days after inoculation, where it also remained well and was killed after 5½ months. There were no pronounced lesions of any kind. Culture tubes inoculated from the heart, liver, and spleen showed no growth.

Hog 1213 was inoculated in the same manner as hog 1212. This hog also remained well and was transferred to exposure pen after 29 days, and was found dead 67 days later. Autopsy showed general condition very poor with numerous minute punctiform hemorrhages in the kidney and numerous large old hog cholera ulcers in the cecum and colon. No cultures were taken in this case.

Hog 1214, the uninoculated pen check on hogs 1212 and 1213, remained well, but on exposure to natural infection became sick in 20 days and died 14 days later. The lungs were firmly adherent to the chest wall; pericardium was thickened and adherent to the heart. The spleen was about normal in size; kidneys pale; on section many hemorrhagic points could be seen in the cortex. On both the large and small intestines many ecchymoses were found.

Filtration B.

The second filtration experiment with the blood of hog 1190 was made with serum from blood which had been kept in the refriger-

TABLE 20.

No. of hog.	Material injected.	Inoculation.		Exposure.		Autopsy.	Remarks.
		Date.	Result.	Date.	Result.		
1208	5 c. c. defibrinated blood hog 1190.	1904, May 2	Sick May 9; killed May 12.	1904.	1904.	Slight lesions.	<i>B. cholerae suis</i> present.
1209	do.	do.	Sick May 9; died May 10.	do.	do.	Hemorrhages and ulcers.	Do.
1269	50 c. c. unfiltered serum hog 1190.	May 25	Died May 31.	do.	do.	Slight lesions.	Do.
1212	47 c. c. filtered serum hog 1190.	May 3	Remained well.	June 1	Killed Nov. 25.	No lesions.	No growth in culture media.
1213	do.	do.	Remained well but stunted.	do.	Died Aug. 7.	Ulcers.	No cultures taken.
1214	Pen check on hogs 1212 and 1213.	(a)	Remained well.	do.	Sick June 21; died July 13.	Hemorrhages.	Do.
1266	50 c. c. filtered serum hog 1190.	May 25	Sick June 9; died July 1.	do.	do.	Slight lesions.	<i>B. cholerae suis</i> not present.
1267	do.	do.	Sick June 10; died July 6.	do.	do.	Ulcers.	
1268	Pen check on hogs 1266 and 1267.	(b)	Sick June 15; died July 27.	do.	do.	No autopsy.	

a Exposed May 3.

b Exposed May 25.

ator for 22 days, and in the incubator for 2 days. The serum was drawn off, as before, and mixed with beef broth, but was in this case filtered through a Berkefeld No. 7 filter instead of a Chamberland "F," as in Filtration A. A culture flask inoculated with the filtrate failed to show the presence of *B. cholerae suis*.

Hog 1269 was injected with 50 c. c. of diluted unfiltered serum from hog 1190, and was found dead 6 days after inoculation. The only distinct lesions which could be found in this hog at autopsy were a severe congestion and partial destruction of the mucosa of the stomach near the pyloric end, and also a swelling and reddening of the ileocecal valve. Notwithstanding the lack of lesions in this hog, *B. cholerae suis* was found in cultures from the various organs.

Hog 1266 was inoculated with 50 c. c. of filtered serum from hog 1190; first symptoms of illness showed in 15 days. The hog became gradually worse, and was found dead 37 days after inoculation. The autopsy revealed two ulcers in the mucous membrane of the stomach, and a number of small grayish erosions in the colon and around the base of the ileocecal valve.

Hog 1267 was injected in the same manner as hog 1266; was noticed to be sick on the sixteenth day; became gradually worse, and died 42 days after inoculation. The autopsy revealed a condition which resembled that found in hog 1266 very closely. There were a number of small yellowish gray ulcers in the colon, and a somewhat larger ulcer at the base of the ileocecal valve.

Hog 1268 was not injected, but placed in the pen with hogs 1266 and 1267 as a check; showed first symptoms of illness 21 days after exposure, became gradually worse, and was found dead 62 days after exposure. Unfortunately decomposition had progressed so far at the time this animal was found that an autopsy could not be satisfactorily made.

The results of the experiments with the blood of hog 1190 show that the blood did not possess any high degree of virulence after filtration, whereas it was decidedly virulent before filtration, since the hogs inoculated with the unfiltered serum, both the diluted and undiluted, died promptly. It would seem, therefore, as if the blood had become

attenuated by the filtration. But this has not been observed with any regularity; in fact, this is the only experiment in which the difference of virulence between the filtered and unfiltered blood was very noticeable. As often as not the animals inoculated with filtered serum contract the disease and die as promptly as those inoculated with unfiltered serum. Examples are not lacking in which the filtered serum produced sickness and death even more promptly than the same serum unfiltered. (See Filtration Experiment No. 8.)

It should be noted that hogs 1212 and 1213, inoculated with the filtered serum, were found to possess quite a marked degree of resistance when exposed to natural infection, whereas the check animal succumbed much more quickly in the exposure pen.

FILTRATION EXPERIMENT No. 7.

The animal furnishing the blood for this experiment, hog 1208, has been already mentioned in connection with Filtration Experiment No. 6, and, as noted there, was etherized when very sick and blood drawn from the heart.

The blood was not defibrinated in this case, but was allowed to clot in the cold, with the exception of 5 c. c., which was defibrinated and injected into hog 1229. The serum was drawn off after 24 hours and 30 c. c. added to 300 c. c. of beef broth. Of this diluted serum 25 c. c. was added to a flask of 400 c. c. of sterilized beef broth and placed in the incubator. To the remainder of the diluted serum 4 or 5 c. c. of a culture of *B. prodigiosus* was added and the whole mixture was then passed through a Chamberland "F" cylinder. The filter was so arranged that the filtration took place from within outward, as shown in plate 2.

A guinea pig, 4692, inoculated with the undiluted, unfiltered serum, died in 16 days with the lesions usually found after such inoculations and cultures of *B. cholerae suis* were obtained from the various organs. The organism obtained in this case was used in many of the experiments with cultures.

The flask of beef broth inoculated with 25 c. c. of the diluted unfiltered serum showed a growth of *B. cholerae suis*. The portion of the filtrate left, after taking out two lots of 50 c. c. each for the inoculation of hogs, was put in the incubator and developed a cloud in 2 days, but neither by microscopic examination nor by the inoculation of a guinea pig was it possible to show the presence of *B. cholerae suis*. *B. prodigiosus* was also shown to be absent from the filtrate. So it is evident from this that the filter was efficacious and that the cloud which developed in the filtrate was due to a contamination occurring during manipulation.

Hog 1229 was inoculated with 5 c. c. of the defibrinated blood of hog 1208 at the time this animal was killed, and showed symptoms of illness 8 days later. The hog grew steadily worse till found dead in the pen 11 days after inoculation, the autopsy showing the lesions of hemorrhagic hog cholera.

TABLE 21.

No. of hog.	Material injected.	Inoculation.		Autopsy.	Remarks.
		Date.	Result.		
1229	5 c. c. defibrinated blood hog 1208	1904.	1904.		
1235	50 c. c. filtered serum hog 1208	May 12	Sick May 20; died May 23	Hemorrhages	<i>B. cholerae suis</i> present.
1236	do	May 14	Sick May 23; died June 20	Hemorrhages and ulcers	No cultures taken.
1237	Pen check on hogs 1235 and 1236	do	Sick May 19; killed May 23	Hemorrhages	<i>B. cholerae suis</i> not present.
		(a)	Sick May 31; killed June 8	do	No growth from organs.

a Exposed May 14, 1904.

Hog 1235 was injected subcutaneously with 50 c. c. of the filtered serum from hog 1208, and placed in a pen with hogs 1236 and 1237. The first symptoms of illness in hog 1235 were noticed 9 days after inoculation; the hog became gradually worse until the thirty-seventh day, when it was found dead. Autopsy revealed a few small areas of broncho-pneumonia; a few ecchymoses on the epicardium; congestion of the kidneys; congestion of the mucosa of the stomach, with one small ulcer; pigmentation of the mucosa of the duodenum; congestion of the mucosa of the jejunum. Mucosa of the ileum was covered by a thick yellowish-gray exudate; the cecum was in the same condition as the ileum; the colon contained many small depressed ulcers, several large necrotic areas, and a few ecchymoses; the mucosa of the rectum was pigmented and sprinkled with ecchymoses; inguinal glands and mesenteric glands were congested. No cultures were made in this case.

Hog 1236 was injected on the same date in precisely the same manner as hog 1235; showed first signs of sickness after 5 days, was very sick on the ninth day, when the hog was etherized and the blood drawn for use in another experiment. (See p. 60). Autopsy revealed numerous ecchymoses in the lungs, spleen, and kidneys; lymph glands generally enlarged, edematous, and hemorrhagic; the mucosa of the stomach was much thickened, and some small hemorrhagic points could be seen in the fundus; the intestines showed similar lesions to those found in hog 1235. Two cultures from the liver and two from the spleen failed to show growth of any kind. A culture from an inguinal gland was also negative.

Hog 1237 was not injected but placed in the pen with hogs 1235 and 1236 at the time these animals were inoculated; became sick after 17 days. The hog grew steadily worse and was etherized when desperately sick on the twenty-fifth day after exposure. The autopsy revealed many dark congested areas in the lungs, ecchymoses on the surface of the heart, the lymph glands were enlarged and dark red. Culture tubes inoculated from heart, liver, and spleen showed no growth of any kind after being in the incubator for a number of days.

It is obvious from this experiment that the filtered blood serum of hog 1208 was highly infectious for other hogs, and that it did not differ in this respect from the unfiltered blood of the same animal, as there was no noticeable difference either in the incubation period or the time of death.

It should be noticed also that in this case, as in the other experiments with filtered blood serum, that the uninoculated check contracted the disease from the animals inoculated with the filtered blood serum. This is a striking and important point in connection with all experiments with filtered blood serum—that is, the animals not only become sick themselves, but they communicate the disease to healthy hogs by association.

FILTRATION EXPERIMENT No. 8.

Hog 1236, the animal furnishing the blood for this experiment was inoculated subcutaneously with the Chamberland filtrate from the blood serum of hog 1208, as already described on page 59.

Hog 1236 was very sick 9 days after inoculation and was then etherized, as already explained in Filtration Experiment No. 7, where the autopsy record is given. As much blood as possible was drawn from the heart and allowed to clot. The day following 35 c. c. of the serum was drawn off and added to 350 c. c. of sterilized beef broth. Of this diluted serum 50 c. c. was put into a flask of about 400 c. c. of sterilized beef broth and placed in the incubator. The rest of the diluted serum was mixed with approximately 5 c. c. of a beef broth culture of *B. prodigiosus* and filtered through a Berkefeld cylinder No. 7. A portion of the filtrate was used for the inoculation of hogs, the rest of the filtrate, about 150 c. c., being placed in the incubator. The culture flask from the unfiltered serum, and the filtrate, both of which were kept in the incubator for several days, failed to show the presence of *B. cholerae suis*, nor could *B. prodigiosus* be demonstrated in the filtrate. Cultures from the liver, spleen, and inguinal gland of hog 1236 also failed to show the presence of *B. cholerae suis*.

Hog 1259 was injected inside of the right thigh with 50 c. c. of diluted unfiltered serum of hog 1236 and placed alone in a pen; became sick after 13 days and showed alternately signs of improvement and signs of returning sickness for 49 days after inoculation, when the animal was killed. The autopsy showed no pronounced lesions of any kind. No cultures were made in this case.

Hog 1261 was injected subcutaneously inside of the thigh with 50 c. c. of Berkefeld filtrate from the blood of hog 1236. The animal was very sick 12 days after inoculation and grew rapidly worse and was killed in a moribund condition 23 days after inoculation. The autopsy showed a few hemorrhages in the heart muscle and numerous hemorrhages in the stomach and cecum. Culture tubes from the lungs, heart, and spleen showed no growth. A sarcina was found in the culture made from the liver.

Hog 1262, injected on same date, with same kind and amount of material as hog 1261, was slightly sick 7 days after inoculation; became gradually worse, and was desperately sick 17 days after inoculation, when it was etherized to obtain blood for other experiments. The autopsy revealed ecchymoses in the lungs, kidneys, spleen, and liver. The inguinal glands were especially large and red. Cultures of *B. cholerae suis* were obtained from the internal organs.

Hog 1260 was not injected, but placed in the pen with hogs 1261 and 1262 as a check; showed first signs of sickness in 16 days, grew steadily worse, and was found dead 24 days after being placed in pen with hogs 1261 and 1262. Autopsy revealed intense congestion of the liver; spleen showed numerous ecchymoses; fundus of the stomach intensely reddened and roughened; the duodenum was thickly sprinkled with hemorrhagic points, and the jejunum showed the same condition. In the ileum were a few hemorrhagic markings; the large intestine showed numerous ecchymoses and erosions, especially in the colon; the lymph glands were enlarged and showed a few ecchymoses. Culture flasks inoculated from the heart and spleen showed no growth.

The results in this experiment show that the filtered blood was virulent, and that it produced in the inoculated hogs a more acute form of disease than that found in the one animal inoculated with the unfiltered blood. The hog, 1259, inoculated with unfiltered blood, was slightly sick 13 days after inoculation, whereas hog 1261, inoculated with the filtered blood, was very sick 13 days after inoculation, and the other

TABLE 22.

No. of hog.	Material injected.	Inoculation.		Autopsy.	Remarks.
		Date.	Result.		
1259	50 c. c. unfiltered serum hog 1236	1904. May 24	1904. Sick June 6; killed July 12	No marked lesions	No cultures taken.
1261	50 c. c. filtered serum hog 1236	do	do	Hemorrhages	No growth in cultures from organs.
1262	do	do	do	do	<i>B. cholerae suis</i> present.
1260	Pen check on hogs 1261 and 1262	(a)	Sick June 1; killed June 10 Sick June 8; died June 17	do	No growths in cultures from organs.

^a Exposed May 24.

hog, 1262, inoculated with filtered blood, was very sick 14 days after inoculation. Again, the course of the disease in the animal inoculated with unfiltered blood was milder in every way than in those animals inoculated with filtered blood. The probability is that the animal that was inoculated with the unfiltered blood possessed greater resisting power than those inoculated with the filtered blood, but the results of the experiment indicate that the blood suffered no material diminution of virulence by passage through the filter.

It should be noted that *B. cholerae suis* was present in one of the hogs inoculated with filtered blood, but not present in the other, nor in the pen check, as determined by the usual methods of examination.

The contagiousness of the disease in hogs inoculated with filtered blood is again shown in this experiment, as the uninoculated hog placed in the pen with the animals inoculated with the filtered blood contracted hog cholera from the inoculated animals as shown by the fact that it remained well until 3 or 4 days after these had shown the first symptoms of disease.

FILTRATION EXPERIMENT No. 9.

Hog 1237, the animal furnishing the blood for this experiment, was not inoculated, but contracted the disease by association with hogs 1235 and 1236, and these hogs were infected by inoculation with filtered serum from hog 1208. (See Filtration Experiment No. 7.) When hog 1237 was desperately sick it was etherized and blood was drawn from the heart. Cultures were made from the liver, lungs, spleen, and lymph glands, and 10 c. c. of the blood was introduced into a 400 c. c. flask of sterile beef broth, but none of these cultures showed a growth, even after incubation for several days. The blood was not defibrinated in this case, but was allowed to clot, and after it had stood in the refrigerator overnight as much serum as possible was drawn off and mixed with beef

broth in the usual proportion of 1 part of serum to 10 parts of broth and filtered through Berkefeld cylinders No. 7, in two portions which were afterwards mixed together. *B. prodigiosus* was not added before filtration in this case. The time required for this filtration was longer than usual for the reason that the serum contained a number of red cells which tended to clog the filters. After taking out the portions to be used for subcutaneous inoculation, as described below, the remainder of the filtrate—about 200 c. c.—was placed in the incubator and found to be cloudy the next day, but careful cultural tests and the inoculation of a guinea pig failed to reveal the presence of *B. cholerae suis*.

Each of 2 hogs, 1296 and 1297, was inoculated subcutaneously with 25 c. c. of the filtered serum and placed in a pen with an uninoculated hog, 1299, as a check. One hog, 1298, was inoculated subcutaneously with 25 c. c. of the diluted unfiltered serum.

Hog 1298, injected subcutaneously inside the thighs with 25 c. c. of diluted unfiltered serum of hog 1237 and placed alone in a pen, showed first signs of illness in 11 days, grew continually worse, and died 32 days after inoculation. Autopsy revealed congestion of the lungs, enlargement and congestion of the liver, spleen, and kidneys, and a number of erosions of the mucosa of the stomach near the pyloric end. A few hemorrhages in the small intestine; two small ulcers at the base of the ileocecal valve and numerous ulcers in the colon. No cultures were taken.

Hog 1296 was inoculated subcutaneously inside of thigh with 25 c. c. of Berkefeld filtrate from hog 1237; placed in pen with hogs 1297 and 1299; showed first symptoms of sickness in 9 days, grew steadily worse and died 18 days after inoculation. The body was too badly decomposed for autopsy or cultures.

Hog 1297, injected on the same date and with the same material and dose as hog 1296, showed first signs of sickness in 8 days; grew gradually worse until 13 days after inoculation, when it was killed. The spleen was greatly enlarged and the kidneys were swollen and pale; the large intestine showed a number of minute punctiform ecchymoses; the lymph glands were enormously enlarged. No cultures were taken in this case.

Hog 1299 was not inoculated but placed in the pen with hogs 1296 and 1297 as a check; showed first signs of sickness in 20 days and grew steadily worse until death, 23 days after exposure. Autopsy revealed a few small hemorrhagic foci in the lungs; numerous punctiform ecchymoses on the surfaces of the heart and kidneys; numerous minute hemorrhagic spots beneath the mucosa of the small intestine, cecum, colon, and rectum. *B. cholerae suis* was obtained in cultures from the heart blood, liver, and spleen.

Hog 1344 was fed the kidneys and spleen of hog 1297 and was placed in a separate pen; was first noticed sick 6 days after the feeding, and was found dead on the thirteenth day. *B. cholerae suis* could not be found in the cultures made from the organs of hog 1344 at the time of autopsy. There was no growth whatever in the tubes inoculated from the liver, spleen, and heart blood; there was a growth consisting of a micrococcus in the culture from the lung. The autopsy showed many ulcers in the cecum and colon, and hemorrhagic lesions on the heart, in the lungs, kidneys, and lymphatic glands.

It will be noted in this experiment that the animals inoculated with the filtered serum both showed symptoms of hog cholera more quickly after inoculation than the hog inoculated with the unfiltered serum. The hogs inoculated with the filtered serum showed symptoms 8 and 9 days, respectively, after inoculation, whereas the hog inoculated with the unfiltered serum did not show symptoms until 12 days after inoculation.

The blood used in these inoculations was free from bacteria of any kind before filtration, and was free from *B. cholerae suis* after filtra-

TABLE 28.

No. of hog.	Material injected.	Inoculation.		Autopsy.	Remarks.
		Date.	Result.		
1298	Diluted unfiltered blood hog 1237	1904, June 9	1904, Sick June 20; died July 11.	Hemorrhages; intestinal ulcers	No cultures taken.
1296	25 c. c. filtered serum hog 1237	do.	Sick June 18; died June 27	No autopsy	Do.
1297	do.	do.	Sick June 17; killed June 22	Hemorrhages	Do.
1299	Pen cheek on hogs 1296 and 1297	do.	Sick June 29; died July 2	do.	<i>B. cholerae suis</i> present.
1344	Fed viscera hog 1237	(a) (b)	Sick June 28; dead July 5.	Hemorrhages; intestinal ulcers	No growth in cultures from organs.

^a Exposed June 9.^b Fed June 22.

tion, though it became contaminated with foreign bacteria through manipulation. Notwithstanding the absence of *B. cholerae suis*, both the filtered and unfiltered serum produced the disease promptly.

That the disease in the animals inoculated with the filtered serum was infectious is proven by the fact that the hog placed in the pen with these animals as a check contracted the disease from association 12 days after the disease had appeared in the inoculated animals, and, moreover, hog 1344, that was fed the viscera of hog 1297, contracted the disease 6 or 7 days after eating the infectious material.

Unfortunately, cultures were not taken in all cases in this experiment, but in those cases where cultures were made some showed the presence of *B. cholerae suis* and some did not. There seemed to be no connection between the presence of that organism and the appearance or severity of the lesions. In hog 1344, the hog that contracted the disease from eating the viscera of hog 1297, the disease developed in about 6 or 7 days, and the animal died in 13 days with marked lesions, and yet *B. cholerae suis* could not be detected in the blood or any of the organs.

The fact that hog 1344 appears to have contracted hog cholera as a result of eating viscera of hog 1297 is also an interesting point in connection with this experiment, as showing that hogs in which disease is produced by injection of filtered blood have the infectious material in their tissues.

FILTRATION EXPERIMENT No. 10.

The blood used in this experiment was obtained from hog 1262, which had been injected inside of the thigh with 50 c. c. of the Chamberland filtrate from the serum of hog 1236. (See Filtration Experiment No. 8.) Hog 1236 was inoculated with the Berkefeld filtrate from 1208 (see Filtration Experiment No. 7),

so hog 1262 received the infectious agent after it had passed successively through a Berkefeld filter, a hog, and then a Chamberland filter.

Hog 1262 was etherized when in a very sick condition, and about 400 c. c. of blood was drawn from the heart, defibrinated, and strained through a layer of absorbent cotton. The blood was then placed in the refrigerator, and 3 days later the serum was drawn off, mixed with beef broth in the proportion of 1 part of serum to 10 parts of beef broth, and filtered in two portions through a Berkefeld cylinder No. 7. *B. prodigiosus* was not added in this case. After removing a portion of the filtrate for the inoculation of hogs the remainder was placed in the incubator, and although this became clouded, *B. cholerae suis* could not be detected either by cultural methods or by the inoculation of guinea pigs, so the cloud in the filtrate was evidently due to contamination during the manipulation necessary in removing the portions for the inoculation of the hogs. Hogs were inoculated with the filtered blood as follows:

Hog 1317 was injected subcutaneously inside of the thigh with 50 c. c. of Berkefeld filtrate from hog 1262; placed in pen with hogs 1318 and 1319; showed first symptoms of sickness in 16 days, grew gradually worse, and died 39 days after inoculation. The autopsy revealed hemorrhages in the lungs, spleen, kidneys, mucosa of the small intestine, and on the surface of the heart; the cecum and colon showed many fine erosions. No cultures were made in this case.

Hog 1318 was injected with the same kind and amount of material as hog 1317; showed first positive signs of illness in 16 days; grew steadily worse, and when in a dying condition was etherized, 30 days after inoculation. Autopsy revealed one or two hemorrhagic foci in the kidneys, and many ulcers in the large intestines, some of them as much as 3 cm. in diameter. *B. coli communis* was obtained in cultures from heart blood.

Hog 1319 was not injected but placed in the pen with hogs 1317 and 1318 as a check. The first positive signs of sickness were noticed on the twenty-fourth day, and the hog was found dead on the thirty-fifth day after exposure. The autopsy revealed a few punctiform hemorrhages on the heart and enlargement of the spleen. The mucosa of the small intestine throughout showed hemorrhagic markings, and the cecum and colon contained many button ulcers.

TABLE 24.

No. of hog.	Material injected.	Inoculation.		Autopsy.	Remarks.
		Date.	Result.		
1317	50 c. c. filtered serum hog 1262.	1904. June 13	1904. Sick June 29; died July 22.	Hemorrhages.	No cultures taken.
1318	do.....	do.....	Sick June 29; killed July 13.	Ulcers	<i>B. coli communis</i> in cultures.
1319	Check on hogs 1317 and 1318.	Exp'd. June 13	Sick July 7; died July 18.	do.....	No cultures taken.

This experiment possesses peculiar interest, from the fact that the animals inoculated with the filtered blood received the infectious agent after it had passed through first a filter, then a hog, then a filter, then a hog, and finally a filter. Further interest might possibly have been added to the experiment if the blood of hog 1261 had been used for inoculation instead of that from 1262; *B. cholerae suis* could not be detected in the unfiltered blood of hog 1261. Still, it was impossible

to foresee this when the choice was made, and, after all, the tests made to determine the presence or absence of *B. cholerae suis* were certainly sufficient. Not only did cultures made from the filtered blood fail to show the presence of *B. cholerae suis*, but the result of the inoculation of 2 guinea pigs, one with the serum immediately after filtration, the other with the filtrate after incubation, also showed that *B. cholerae suis* was absent.

In order to determine whether this very surprising power of blood to induce hog cholera, even though no hog-cholera bacilli were present in it, was peculiar to the strain of disease with which we had been working, or whether it was a property possessed by all outbreaks of acute hog cholera, material was obtained from a number of separate and distinct outbreaks of that disease. Experiments were instituted along the same general lines as were followed in those which have been previously described. Only those outbreaks were chosen which exhibited undoubted characteristics of hog cholera, such as contagiousness and high mortality among the affected animals, and which also produced the lesions and symptoms usually found in animals attacked by that disease. A short description of each outbreak precedes each set of experiments.

EXPERIMENTS WITH BLOOD FROM HERD II.

The outbreak of hog cholera from which this strain of disease was obtained appeared about the middle of June, 1903, in a herd in southwestern Iowa. At the time the disease appeared the herd consisted of 85 small pigs and 20 brood sows. The symptoms exhibited were those ordinarily seen in outbreaks of acute hog cholera. The lesions found at autopsy were of the hemorrhagic type, the intestinal ulcerations being absent in practically all of the animals which were examined. The disease proved very virulent. Of the 85 pigs only 2 survived, and of the 20 brood sows only 6 survived. On July 1, when the disease was at its height, a sick hog was killed in order to obtain blood for use in our experiments. This animal presented at autopsy typical lesions of hemorrhagic hog cholera. Hog 816, which was injected subcutaneously with the blood of this hog, was shipped immediately by express to Washington, and served as the starting point for all of the experiments which are described under the head of herd II disease. This disease was found to be readily communicable from diseased to healthy animals by subcutaneous injections of diseased blood, and the symptoms and lesions exhibited by the inoculated animals were quite typical of acute hog cholera. The farm on which this outbreak occurred was located in the same township as herd I, but about 9 miles distant by road. No connection between the two outbreaks could be traced.

FILTRATION EXPERIMENT NO. 11.

Hog 852, the animal which furnished blood for this experiment, was injected subcutaneously with 5 c. c. of blood from hog 847, the latter animal being sick at the time as a result of a previous injection of diseased blood from herd II. Hog 852 was first noticed to be sick 7 days after inoculation; became rapidly worse, and was etherized 2 days later to obtain blood for Filtration Experiment No. 11. The autopsy revealed rather slight lesions, the only characteristic changes being found in the cecum, which was thickly sprinkled with small recently formed ulcers. The defibrinated blood of hog 852 was allowed to stand in the ice box for 3 days, when a considerable amount of clear serum had separated from the red cells. The serum was withdrawn by means of a sterile pipette and mixed with sterile normal salt solution. This diluted serum was divided into three portions. One portion was filtered through a Berkefeld laboratory cylinder No. 7, a second portion through a Chamberland cylinder "B," and the third portion was left unfiltered.

Cultures were made from the filtrate and from the unfiltered serum in the following manner: Two flasks containing 400 c. c. of neutral beef broth were inoculated with 25 c. c. of each of the filtrates, and a third flask was inoculated with $3\frac{1}{2}$ c. c. of the undiluted unfiltered serum. A rabbit was injected subcutaneously at the same time with 3 c. c. of the undiluted and unfiltered serum. Inasmuch as the filtered serum represented a dilution of 1 to 10, this rabbit received the equivalent of 33 c. c. of the filtered serum. The rabbit remained perfectly well for more than 6 months after inoculation. The cultures from the Chamberland "B" filtrate and from the unfiltered undiluted serum both developed a cloud at the end of 48 hours. The culture from the Berkefeld filtrate, however, remained sterile. A microscopic examination of the growths in the cultures from the unfiltered serum and from the Chamberland "B" filtrate showed in both instances a pure culture of a micrococcus. A guinea pig inoculated subcutaneously with $\frac{1}{2}$ c. c. of the culture from the Chamberland filtrate remained alive for a month after inoculation, dying then as a result of an epidemic, not due to *B. cholerae suis*, which was prevalent among our guinea pigs at that time.

Inasmuch as the culture from the unfiltered serum did not disclose the presence of *B. cholerae suis*, and as the rabbit inoculated with 3 c. c. of the same serum undiluted showed no signs of disease as a result, we seemed to be justified in concluding that *B. cholerae suis* was absent from the serum of hog 852 at the time the blood was used for filtration. After filtration the filtered serum, together with a certain amount of the diluted unfiltered serum, was kept on ice overnight and used the next day for inoculation.

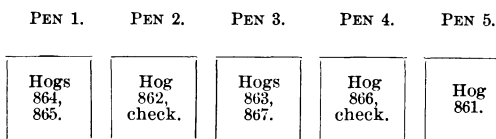
Hog 861 received 22 c. c. of the diluted, unfiltered serum, hogs 863 and 867 received each 22 c. c. of the Berkefeld filtrate, hogs 864 and

TABLE 25.

No. of hog.	Material injected.	Inoculation.		Autopsy.	Remarks.
		Date.	Result.		
861	22 c. c. unfiltered serum hog 852.	1903. Aug. 18	Sick Aug. 26; died Sept. 8.	Hemorrhages and ulcers.	No cultures taken.
862	22 c. c. filtered serum hog 852, Berkefeld.	do.	Sick Aug. 27; died Sept. 13.	do.	Do.
864	do.	do.	Sick Aug. 27; died Sept. 19.	do.	Do.
865	22 c. c. filtered serum hog 852, Chamberland B.	do.	Sick Aug. 24; killed Sept. 1.	do.	Do.
862	Neutral beef broth; syringe check on hogs 864 and 865.	do.	Remained well.	do.	Removed from experiment Oct. 1, 1903.
866	Neutral beef broth; syringe check on hogs 863 and 867.	do.	do.	do.	Do.

865 received each 22 c. c. of the Chamberland "B" filtrate, and hog 862 received 22 c. c. of neutral beef broth, the same syringe being used for the injection of hogs 864 and 865. Hog 866 was also injected with beef broth, and the syringe employed in this case was afterwards used for the injection of hogs 863 and 867. These injections were made in order to determine whether or not the syringes were free from the virus of hog cholera.

The pens used were 6 feet apart and so constructed that communication from one to the other was impossible except through the air. After thorough disinfection of these pens the inoculated hogs were placed in them in the manner indicated by the accompanying diagram:



Hog 861 was inoculated with diluted unfiltered serum of hog 852. The first signs of sickness appeared in 8 days, and from this time on the animal became steadily worse, dying 21 days after inoculation. The ventral surface of the body was reddened; the inguinal glands were enlarged, edematous and hemorrhagic. In the lungs several areas of red collapse were found; the spleen was enlarged and engorged with blood; the kidneys were sprinkled with punctiform hemorrhages; both the large and small intestines exhibited numerous ecchymoses on their mucous surfaces, and extensive ulceration was found in the colon. No cultures were made.

Hog 863 was inoculated with the Berkefeld filtrate from serum of hog 852. The first symptoms of sickness appeared in 9 days; death occurred in 26 days. The autopsy presented numerous extravasations on the surface of the heart; some areas of red collapse in the lungs; spleen enlarged and engorged with blood; kidneys thickly dotted with punctiform ecchymoses. Numerous small hemorrhages and some erosions in the mucosa of the cecum and colon.

Hog 867 was inoculated with the same material as hog 863. The first symptoms of sickness appeared in 9 days. The animal remained quite sick, and died 32 days after inoculation. The autopsy revealed enlarged and edematous inguinal glands, the cortical portions of which were hemorrhagic; numerous minute petechiæ were found on the heart muscle; spleen was enlarged and engorged with blood; kidneys were sprinkled with punctiform hemorrhages, and there was extensive ulceration of the mucous membrane of the cecum and colon.

Hog 864 was injected with the Chamberland filtrate from serum of hog 852. The first symptoms of disease appeared in 6 days; in 14 days the hog was very sick and weak, could hardly walk, and refused food. In order to obtain blood for another experiment, the hog was etherized at this time and blood was drawn from the heart and defibrinated. The autopsy revealed no lesions at the seat of injection. The cortices of the kidneys contained a few hemorrhagic points, and there was extensive ulceration of the cecum and colon.

Hog 865 was injected with the same material as hog 864. The first symptoms of illness appeared in 6 days; the hog became rapidly worse and died 14 days after inoculation. The autopsy revealed hemorrhagic lesions and also a number of recently formed ulcers in the large intestine.

Hog 862 was injected subcutaneously with 22 c. c. of neutral beef broth as a check on hogs 864 and 865. This hog remained perfectly well and was removed from the experiment after 6 weeks.

Hog 866 was injected subcutaneously with 22 c. c. of neutral beef broth as a check on the syringe used for the injection of hogs 863 and 867. This hog also remained perfectly well and was removed from the experiment after 6 weeks.

This experiment is in entire accord with those previously described. Checks were not placed in the pens with the injected hogs, but the fact that the two check animals in pens adjoining, under conditions entirely similar to the inoculated hogs, remained well, seemed to furnish good evidence that the infection was derived from the material injected. This conclusion is strengthened by the fact that the results here obtained agree with those already reported. That the disease communicated by this filtered serum possessed the infectious character of hog cholera is shown by the injections of the blood of hog 864 which are described in the following experiment.

FILTRATION EXPERIMENT No. 12.

This experiment was undertaken in order to determine whether or not the blood of animals which were inoculated with filtered serum was infectious for other animals in the same degree as that obtained from hogs injected with unfiltered blood. Similar experiments will be found elsewhere.

The record of hog 864 has already been given in Filtration Experiment No. 11 and need not be repeated here. The blood was drawn, defibrinated, and kept in the refrigerator for 3 days, when 20 c. c. of the clear serum was withdrawn by means of a sterile pipette and mixed with 200 c. c. of sterile physiological salt solution. This diluted serum was divided into two portions. One portion was filtered through a Chamberland "B" cylinder, the other portion was left unfiltered. Twenty-five cubic centimeters of the Chamberland filtrate was added to 400 c. c. of neutral beef broth and placed in the incubator. At the same time 4 c. c. of the unfiltered and undiluted serum was added to a similar flask. Moreover, a rabbit was inoculated subcutaneously on the same date with 3 c. c. of the same unfiltered undiluted serum.

The bouillon culture from the unfiltered and undiluted serum developed a pure growth of a small motile organism resembling *B. cholerae suis*. The identification of this culture was not made complete. The rabbit which was inoculated at the same time the cultures were made

died on the sixth day after inoculation and from its organs a pure culture of a small motile organism was obtained. This organism was identical with *B. cholerae suis* in every way except in its fermentative characters, no gas being produced in glucose bouillon. It probably represented a rare variety of *B. cholerae suis* which does not produce a gaseous fermentation of glucose. The culture from the filtrate remained sterile. The portions of filtered and unfiltered serum remaining after the cultures were made were used for the injection of hogs, each hog being placed in a separate pen.

Hog 906 was injected with 22 c. c. of the diluted unfiltered serum of hog 864; showed signs of illness on the seventh day and died 17 days after inoculation. The lesions were quite similar to those found in hog 905. There were extensive hemorrhages, together with ulcers in the large intestine.

Hog 905 was injected with 22 c. c. of the filtered serum; showed symptoms of illness on the seventh day, became gradually worse, and was found dead 17 days after inoculation. The autopsy revealed hemorrhagic lesions and also extensive ulceration of the colon.

Hog 907 was injected with 22 c. c. of neutral beef broth, the syringe employed for this injection being subsequently used for the injection of hog 905. This hog remained perfectly well and was removed from the experiment after 26 days.

TABLE 26.

No. of hog.	Material injected.	Inoculation.		Autopsy.	Remarks.
		Date.	Result.		
906	22 c. c. unfiltered serum hog 864.	1903. Sept. 5	1903. Sick Sept. 12; died Sept. 22.	Hemorrhages and ulcers.	No cultures taken.
905	22 c. c. filtered serum hog 864.	do....	do.....	do.....	Do.
907	Sterile beef broth; syringe check on hog 905.	do....	Remained well...		Removed from experiment Oct. 1, 1903.

This experiment is interesting, not only as a corroboration of the results previously obtained with filtered serum, but also because it shows that the blood of hog 864, made sick by an inoculation of filtered serum, was in its turn infectious when filtered and injected into another hog.

FILTRATION EXPERIMENT No. 13.

This experiment was undertaken primarily for the purpose of checking the results obtained in Filtration Experiment No. 12. It was also intended to broaden the experiment somewhat by continuing the passage of the virus through a number of hogs, the blood being filtered previous to each inoculation. As will be seen from the records given below, we were only able to make two passages of the virus, owing to the fact that the second animal inoculated recovered from the disease, which was induced by the injection.

Hog 935 was injected subcutaneously with 7 c. c. of defibrinated blood from hog 911; the latter hog being affected at the time with the herd II disease. Hog 935 became desperately sick and was etherized 9 days after inoculation, and about 300 c. c. of blood was drawn and

defibrinated for use in the experiment; 7 c. c. of this was used to inject hog 942 (see below). One day later the serum, which had separated from the red cells in the defibrinated blood of hog 935, was withdrawn by means of a sterile pipette and diluted with 10 parts of a sterile physiological salt solution. The serum contained a small number of red cells. After dilution the serum was passed through a Chamberland cylinder "F." Of the filtrate obtained in this way, 50 c. c. was used for making cultures, 25 c. c. being added to each of two flasks containing sterile neutral beef broth. The cultures were placed in the incubator, where they remained perfectly clear and free from growth in so far as ordinary bacteriological methods could detect. Portions of the remaining filtered serum were placed in the ice box in several different flasks. Two days later 2 rabbits were inoculated with the filtrate, each rabbit receiving subcutaneously 22 c. c., a similar amount being used later for the inoculation of hog 973. Neither of the rabbits sickened as a result of inoculation and were alive and well 1 year later. A guinea pig injected with the defibrinated blood of hog 935, not filtered, died 6 days after inoculation and cultures revealed the presence of *B. cholerae suis*.

Hog 972 was injected with sterilized beef broth, and the same syringe was afterwards used to inject hog 973 with filtered blood. The 2 hogs 972 and 973 were placed in separate disinfected pens. Hog 972 remained perfectly well as a result of the injection, and was injected 47 days later with 8 c. c. of defibrinated blood from herd I disease. Twelve days after the latter injection this animal was desperately sick and was etherized. The autopsy revealed lesions of hemorrhagic hog cholera. The death of this animal as a result of the blood inoculation shows that it possessed no immunity, and therefore that the syringe used for the injection of 973 contained no infectious material.

Hog 973, which was injected with the filtered serum of hog 935, was slightly sick in 7 days; became worse, and died 16 days after injection, just as preparations were being made to draw blood for the continuance of the experiment as previously outlined. Immediately after death blood was drawn from the heart and defibrinated; a small portion was used for the injection of hog 981, and the remainder placed on ice. The autopsy revealed slight reddening of the ventral surface of the body. All of the lymphatic glands exhibited hemorrhagic lesions. There were hemorrhages on the surface of the heart and in the lungs and the spleen. The liver was mottled and exhibited numerous minute hemorrhagic points. The kidneys were swollen and the cortices were sprinkled with well-defined punctiform hemorrhages which varied from $\frac{1}{4}$ to 1 mm. in diameter. The stomach exhibited numerous ecchymoses on its mucous surface. The mucosa of the small intestine contained numerous hemorrhages, and a few extravasations were also noted on the serous covering of that portion of the bowel. The cecum and colon presented numerous small ecchymoses on the mucous surface and a few on the serous surface also. In the colon a number of thick ulcers and necrotic areas were found.

Hog 942 was injected with unfiltered defibrinated blood from hog 935 for the purpose of testing the pathogenic power of that blood, and also in order to preserve the herd II disease in the event that hog 986, which was later injected with the filtered serum, failed to sicken. In 7 days hog 942 was found to be very sick and was killed in order to obtain blood for future experiments. The autopsy disclosed the lesions of subacute hog cholera.

The blood of hog 973 was kept on ice for 3 days, at which time a considerable amount of clear serum had separated. A portion of this clear serum was diluted with physiological salt solution, 1 part of serum to 10 parts of salt solution, and filtered through a Chamberland "F" cylinder. Hog 986 was injected subcutaneously with 22 c. c. of

the filtrate. The syringe which was used for injecting hog 986 was previously used to inject hog 972 with sterile bouillon in order to test the sterility of the syringe, as was done in previous cases.

Hog 986 became sick in 12 days and remained so for 7 or 8 days. After 25 days, recovery appeared to be complete. The animal then remained well for 31 days, when it received an injection of blood from hog 1018 carrying herd I disease. No illness followed this disease-blood injection, and the animal 2 months and 5 days later was placed in the exposure pen, together with hogs 1132 and 1133. The two latter animals served as checks on the virulence of the disease in the exposure pen. Hog 986 survived this exposure, although 1132 and 1133, which were untreated nonimmunes, succumbed, the former in 13 days and the latter in 10 days.

Hog 972, used as a syringe check on hog 973, as previously stated, was, after 19 days, used as syringe check for hog 986. The record of this animal in connection with hog 973 has already been given. No illness followed the injection of the sterile bouillon, and a subsequent injection with diseased blood proved that the hog possessed no immunity, thus indicating that the syringe used for the injection of hog 986, as well as the one used for injecting hog 973, was free from the virus of hog cholera when used in those experiments.

Hog 981, which was injected with defibrinated blood from hog 973, was very sick in 13 days and was killed to obtain blood for other experiments. The autopsy revealed the lesions of hog cholera.

TABLE 27.

No. of hog.	Material injected.	Inoculation.		Autopsy.
		Date.	Result.	
942	7 c. c. defibrinated blood hog 935.....	1903. Oct. 5	1903. Sick Oct. 12; killed Oct. 12..	Hemorrhages and ulcers..
973	22 c. c. filtered serum hog 935	Oct. 12	Sick Oct. 26; died Oct. 28....	Do.
981	Defibrinated blood hog 973	Oct. 23	Sick Nov. 5; killed Nov. 5..	Do.
986	22 c. c. filtered serum hog 973	Oct. 31	Sick Nov. 12; ^a recovered.	
972	Not inoculated; syringe check on hogs 973 and 986.	{Oct. 12 Oct. 31}	Remained well.	

^a This hog was subsequently exposed to virulent hog cholera, but remained well.

While it is to be regretted that the experiment could not be carried further, we obtained from it a confirmation of the results noted in Filtration Experiment No. 12. The blood of a hog made sick by an injection of filtered serum is in turn infectious for other animals after filtration. The absence of *B. cholerae suis* from the filtered blood of hog 935 was demonstrated by culture tests as well as by the inoculation of rabbits. The death of hog 973 as a result of an injection with that serum and the proven infectiousness of the blood of the latter animal serve therefore as additional proofs of the production of disease by serum not containing *B. cholerae suis*.

EXPERIMENTS WITH BLOOD FROM HERD III.

At the time of the outbreak in May, 1904, this herd consisted of 120 old hogs and approximately 200 spring pigs. As a result of the disease, about 170 of the pigs succumbed, but only 8 old hogs died, although the entire herd was exposed to the infection. It would seem, therefore, that this outbreak was not as virulent as some others from which we have obtained blood.

Autopsies on several pigs which had died showed the lesions usually found in outbreaks of acute hog cholera. The origin of the disease in this herd could not be traced; it certainly had no connection with herds I and II, as the latter were separated many miles from herd III, and, in addition, the outbreaks occurred in these herds nearly a year before the disease appeared in herd III.

FILTRATION EXPERIMENT No. 15.

The blood used in this experiment was obtained from hog 169, one of a series of animals carrying the herd III disease. This hog was etherized when very sick and blood drawn in the usual manner. The autopsy showed enlarged inguinal glands; patches of congestion in the lungs; grayish mottling of the liver; spleen greatly enlarged, very dark, and soft; kidneys rather pale with a few punctate hemorrhages; mucosa of cecum covered with a thick, firm, somewhat gelatinous exudate, but no ulcers. Cultures from heart blood and spleen showed *B. cholerae suis*.

The defibrinated heart blood of hog 169 was filtered through absorbent cotton, and placed on ice overnight. At the end of 20 hours the clear serum was drawn off with a sterile pipette. To 30 c. c. of this serum was then added 300 c. c. of sterile beef broth and 3 c. c. of a suspension of *B. prodigiosus*, and the whole passed through a Chamberland "F" cylinder.

Portions of the diluted serum, before and after filtration, were used for the inoculation of hogs.

The portion of diluted filtered serum remaining, about 225 c. c., was set on one side and showed clouding at the end of 48 hours; a microscopical examination showed no motile organism, and agar plates made from this filtrate failed to show the presence of *B. cholerae suis* or *B. prodigiosus*.

Hog 180 was injected subcutaneously with 25 c. c. of diluted unfiltered blood of hog 169; was first noticed sick on the sixth day and became gradually worse; died on the seventeenth day after inoculation. Autopsy showed lymphatic glands generally enlarged and dark; slight congestion of the lungs; spleen somewhat enlarged, very dark, and very soft; kidneys somewhat pale and mottled, with a few scattered punctate hemorrhages; mucosa over the pyloric end of the stomach intensely reddened; numerous small, well-defined ulcers in cecum and colon. Cultures from heart blood and spleen showed pure growths of *B. cholerae suis*.

Hog 177 was injected subcutaneously with 25 c. c. of diluted filtered serum from hog 169 and placed in a pen with hogs 178 and 179; was first noticed sick on the seventh day, continued very sick, and died 14 days after inoculation. Autopsy showed reddening of skin over throat; inguinal glands enlarged and reddened; lungs thickly studded with small, irregular patches of congestion; spleen much enlarged, dark, and very soft; kidneys congested and thickly studded with small, punctate hemorrhages; small intestine congested and mucosa, thickly studded with small, hemorrhagic points; hemorrhagic patches in mucosa of cecum near ileocecal valve; mucosa of large intestine very hemorrhagic. Cultures from heart blood and spleen showed pure growths of *B. cholerae suis*.

Hog 178 was injected subcutaneously with 25 c. c. of diluted, filtered serum from hog 169; was first noticed sick on the seventh day; became very sick, and died 17 days after inoculation. Autopsy showed inguinal glands reddened; lungs slightly congested; spleen enlarged, dark, and very soft; kidneys somewhat pale and mottled, with a few scattered, punctate hemorrhages; mucosa over pyloric end of

TABLE 28.

No. of hog.	Material injected.	Inoculation.		Autopsy.	Remarks.
		Date.	Result.		
180	23 c. c. unfiltered serum hog 169.....	1904	1904	Hemorrhages and ulcers.....	<i>B. cholerae suis</i> present.
177	25 c. c. filtered serum hog 169.....	July 27	Sick Aug. 2; died Aug. 13	Hemorrhages.....	Do.
178	Check; exposed to hogs 177 and 178.....	do	Sick Aug. 3; died Aug. 10	Hemorrhages and ulcers.....	Do.
179	Check; exposed to hogs 177 and 178.....	do	Sick Aug. 3; died Aug. 13	do.....	Do.
198	5 c. c. defibrinated tail-blood hog 178.....	(a)	Sick Aug. 11; died Aug. 27	Hemorrhages; beginning ulceration.....	Do.
199	Fed viscera of hog 178.....	Aug. 10	Sick Aug. 18; died Aug. 24	Hemorrhages; beginning ulceration.....	Do.
c 205	Exposed in pen with hog 199.....	(b)	Sick Aug. 16; died Aug. 18	Hemorrhages; necrotic enteritis.....	Do.
208	20 c. c. unfiltered urine from hog 198.....	(d)	Remained well.....	Hemorrhages.....	Do.
		Aug. 24	Sick Aug. 26; died Aug. 31	Hemorrhages.....	Do.

a Exposed July 27.

b Fed August 13.

c Hog 205 was placed in pen with hog 199 on August 16 as soon as the latter animal showed symptoms of sickness, and remained perfectly well; was transferred to exposure pen on September 5 and died 14 days later with hemorrhagic lesions and button ulcers.

d Exposed August 16.

stomach much congested; cecum and large intestine showed early ulceration, covered with a thick, tenacious, dirty-yellow exudate. Cultures from heart blood and spleen showed pure growths of *B. cholerae suis*.

Hog 179 was exposed in the pen with hogs 177 and 178 on the day these were inoculated; was first noticed sick on the fifteenth day; continued very sick, and died 31 days after being placed in pen with hogs 177 and 178. Autopsy showed some reddening of the skin over the thorax and abdomen; inguinal glands dark and swollen; lungs showed slight congestion in the cephalic lobes; mucosa over the pyloric end of stomach intensely reddened; spleen slightly enlarged, dark, and soft; cecum and colon showed large, shallow, well-defined ulcers, covered with a thick, yellowish exudate; mesenteric glands dark and swollen. Agar cultures from heart blood and spleen showed pure growths of the *B. cholerae suis*.

Hog 198 was injected subcutaneously with 5 c. c. of defibrinated tail blood from hog 178 and placed in a separate pen; showed first symptoms of sickness on the eighth day; became gradually worse, and died on the fourteenth day after inoculation. Autopsy showed slight reddening of skin over throat and upper part of thorax; inguinal glands dark and swollen; large patches of congestion in lungs; spleen considerably enlarged, dark, and soft; small hemorrhagic points on auricular appendages; mucosa over pyloric end of stomach considerably reddened; kidneys swollen and congested and thickly studded with small punctate hemorrhages; serous surface of small intestine thickly studded with small hemorrhages, which showed also in mucosa; mucosa of cecum showed small hemorrhagic points, and had the appearance of beginning ulceration at the ileocecal valve. Cultures from heart blood, spleen, and urine showed *B. cholerae suis*.

Hog 199 was fed viscera of hog 178; showed first symptoms of sickness on the third day after eating the viscera, became rapidly worse, and died on the fifth day after being fed. Autopsy showed inguinal glands somewhat enlarged and reddened; a few patches of congestion in the lungs; spleen considerably enlarged, dark, and soft; kidneys congested, with a few small punctate hemorrhages; mucosa of stomach intensely reddened; mucosa of cecum and colon showed beginning, diffuse necrosis. Cultures from heart blood and spleen showed *B. cholerae suis*.

Hog 205 was placed in the pen with hog 199 three days after the latter was fed viscera. This animal remained perfectly well; was transferred to exposure pen after 20 days, and died 14 days later. Autopsy showed inguinal glands reddened; small hemorrhagic areas and large patches of congestion in lungs; spleen slightly enlarged and dark; mucosa of stomach slightly reddened; kidneys congested and mottled; intestines showed extensive diffuse ulceration of mucosa and some well-marked button ulcers; mesenteric and lumbar glands red and swollen. Agar cultures from heart blood and spleen showed *B. cholerae suis*.

Hog 208 was injected subcutaneously with 20 c. c. of unfiltered urine from hog 198; showed first symptoms of sickness on second day; became rapidly worse, and died on the seventh day after receiving the injection. Autopsy showed the inguinal glands much enlarged and reddened; lungs thickly studded with hemorrhagic patches; spleen greatly enlarged, very dark, and soft; kidneys congested, with scattered punctate hemorrhages; mucosa over pyloric end of stomach intensely reddened; mesenteric glands reddened, and the mesenteric vessels engorged. Cultures from heart blood and spleen showed *B. cholerae suis*.

Although the filtered blood showed clouding at the end of 48 hours, agar plates made from the cloudy material failed to show either *B. cholerae suis* or *B. prodigiosus*, and the conclusion seems justifiable that *B. cholerae suis* could not have been present in the filtrate at the time it was inoculated.

The hogs inoculated with the filtered serum became sick and died rather more promptly than the hog inoculated with the unfiltered blood. The check became sick and died, evidently from association with the animals inoculated with the filtered blood, and a hog inoculated with tail blood from one of the filtered-serum hogs also died promptly as a result of the injection. In this experiment a hog was also inoculated with urine, and became sick and died very promptly. *B. cholerae suis* was found in all the hogs.

These results confirm those obtained in similar experiments.

FILTRATION EXPERIMENT No. 16.

Hog 1348, which furnished the blood for this experiment, was inoculated June 24 with 5 c. c. of defibrinated blood from hog 1345, sent in from the West, and this hog had in turn been inoculated with blood from a sick hog carrying the herd III disease. Hog 1348 was etherized when very sick, July 9, and blood drawn from the heart; about 650 c. c. was obtained; this was defibrinated and a small amount used to inject hog 1368. The autopsy on hog 1348 showed both kidneys to contain numerous punctiform ecchymoses in the cortical portions. *B. cholerae suis* was obtained in cultures from various organs.

The rest of the blood, after taking out the amount used to inoculate hog 1368, was kept in the refrigerator for 2 days, when the serum was drawn off and mixed with sterilized beef broth in the usual proportion of 1 part of serum to 10 parts of beef broth and about 5 c. c. of a 24-hour culture of *B. prodigiosus* was added.

Just before the addition of the *prodigiosus* culture 25 c. c. of the diluted serum was added to a flask of sterile beef broth and placed in the incubator. A second portion of 25 c. c. was used for the injection, subcutaneously, of hog 1373. The remainder of the diluted

serum containing *B. prodigiosus* was filtered through a Chamberland "F" cylinder. A part of the filtered serum obtained in this way was injected into hogs 1371 and 1372 subcutaneously, 25 c. c. into each, and these were placed in the pen with the uninoculated hog 1370 as a check. The rest of the filtrate, as well as the culture made from the unfiltered serum, became cloudy. An examination revealed the presence of *B. cholerae suis* in the culture from the unfiltered serum, while the cloudiness in the filtered serum was caused by the growth of a micrococcus which appeared to be present in pure culture, evidently accidentally introduced after filtration. In order to check the bacteriological examination, guinea pig 4828 was inoculated subcutaneously with $\frac{1}{2}$ c. c. of the culture from the unfiltered serum, and guinea pig 4827 was inoculated in the same manner with $\frac{1}{2}$ c. c. of the filtered serum which had become clouded in the incubator.

As was expected, guinea pig 4828 became sick, and died at the end of 13 days, with the lesions usually found in such cases, and *B. cholerae suis* was obtained from the different organs. Much to our surprise, however, guinea pig 4827 was found dead 23 days after inoculation, and *B. cholerae suis* was also obtained in cultures from the organs. This guinea pig, 4827, had been placed by oversight in the same cage with guinea pig 4828, which, as previously explained, was inoculated with the culture from the unfiltered blood. Inasmuch as guinea pig 4827 lived 23 days after inoculation, a much longer time than is usual in the case of guinea pigs inoculated with *B. cholerae suis*, and that it was in the same cage with guinea pig 4828, we believe the contagion was derived from the last-named guinea pig and not from the filtered serum with which guinea pig 4827 was inoculated. We must admit, however, that this experiment is not without objection, owing to the presence of *B. cholerae suis* in the tissues of guinea pig 4827.

Hog 1368 was injected subcutaneously inside of left thigh with 5 c. c. of defibrinated blood of hog 1348 and placed alone in a pen. In 9 days the animal showed some symptoms of illness, consisting of scouring and loss of appetite, but was otherwise fairly well. Twelve days after inoculation it refused food, was scouring badly, was spiritless, and uncertain on its feet; was etherized at this time, blood drawn from the heart and defibrinated, and 5 c. c. used to inject hog 1409. The autopsy showed numerous ecchymoses in the lungs; a few punctiform ecchymoses in the heart; hemorrhagic lobules in the liver; a large number of small ecchymoses in the kidneys; minute hemorrhagic points in both the small and large intestines. The bladder also showed a few small diffuse hemorrhagic spots in the mucosa. No cultures were taken.

Hog 1373, inoculated with the diluted blood, unfiltered, became slightly sick 7 days after inoculation, and grew steadily worse until the animal was etherized 11 days after inoculation, and 5 c. c. of the defibrinated blood was used to inoculate subcutaneously hog 1413. The autopsy on hog 1373 showed hemorrhagic lesions, and there were also small ulcers in the large intestine, particularly numerous in the cecum.

Hog 1371 was inoculated with filtered serum and placed in a pen with hogs 1372 and 1370; became sick 12 days after inoculation, with loss of appetite, apathy, staggering, and died 14 days after inoculation. The autopsy showed a large number of hemorrhagic lesions in the kidneys and other organs; also extensive ulceration in the cecum and colon, particularly in the region of the ileocecal valve. Cultures showed the presence of *B. cholerae suis* in the tissues.

Hog 1372, the other hog inoculated with the filtered serum, repeated the history of hog 1371 in every respect; became sick on the same day, died on the same day, and had the same lesions. Cultures of *B. cholerae suis* were also obtained from the organs.

Hog 1370 was injected with 25 c. c. of sterile beef broth, using the same syringe as was afterwards employed to inject the filtered serum, in order to serve as a check on the sterility of the syringe, and of the pen; became sick 4 days after the appearance of symptoms in the animals inoculated with filtered serum, and was found dead 26 days after being placed in the pen with hogs 1371 and 1372. The autopsy revealed the lesions of hemorrhagic hog cholera, besides ulcers in the cecum in various stages of development, and *B. cholerae suis* was obtained in cultures from the various organs.

TABLE 29.

No. of hog.	Material injected.	Inoculation.		Autopsy.	Remarks.
		Date.	Result.		
1368	5 c. c. defibrinated blood hog 1348.	1904. July 9	1904. Sick July 18; killed July 21.	Hemorrhages.	No cultures made.
1373	25 c. c. diluted unfiltered serum hog 1348.	July 11	Sick July 18; killed July 22.	do	Do.
1371	25 c. c. filtered serum hog 1348..	do ...	Sick July 23; died July 25.	do	<i>B. cholerae suis</i> present.
1372	do	do	do	do	Do.
1370	Pen check on hogs 1371 and 1372.	Ex p'd July 11	Sick July 27; died Aug. 6.	do	Do.

The results of this set of inoculations show that the hogs injected with the filtered blood became sick and communicated disease to the check animal. The filtered-blood hogs showed the presence of *B. cholerae suis* in their tissues, although culture tests failed to reveal the presence of this organism in the material used for injection; and, while it is true that the guinea pig inoculated with the same material, contrary to all previous and subsequent experience, died and showed *B. cholerae suis* in the tissues, it is probable that infection in this case was accidental.

FILTRATION EXPERIMENT No. 17.

The blood used in this experiment was taken from hog 1458, which was the eighth in a series of animals starting with hog 1345. Hog 1345, as already stated on page 74, was sent in from Iowa after having been inoculated with the blood of an animal suffering from a spontaneous attack of hog cholera. In this series the disease was conveyed from one animal to the next by injection of defibrinated blood. Since none of the hogs of the series were inoculated with the filtered blood, it is not necessary to go particularly into the course of the disease in any of them previous to hog 1458; suffice it to say that they all suffered from undoubted hog cholera, as shown by autopsy in every case and by the fact that the blood of each of the animals proved infectious when injected subcutaneously into others.

Hog 1458, the animal furnishing the blood in this case, as stated, was inoculated September 8, 1904, with 5 c. c. of the defibrinated blood of hog 1452, and was etherized when very sick, 8 days after inoculation. The blood was drawn from the heart, and a small amount, 5 c. c., was used to inoculate hog 1465; the rest was left in the refrigerator for 12 days. Serum was drawn off, diluted with the usual

proportion of beef broth, and inoculated with about 2 or 3 c. c. of a suspension of *B. prodigiosus* in beef broth, and passed through a Chamberland "F" cylinder. The filtrate was then kept in the incubator for 4 days and remained clear.

In this experiment the effects of freezing and thawing upon the virulence were tried. One portion of the filtered serum, 50 c. c., was distributed into 6 test tubes and frozen solid in a mixture of ice and salt and kept frozen for 15 minutes, thawed out in warm water, and again frozen and kept frozen for 15 minutes. The contents of these tubes were poured together after thawing and 22 c. c. injected into each of 2 hogs, 1520 and 1521, which were placed in a pen with an uninoculated check hog, 1522. A culture of *B. cholerae suis* was also exposed, in the same manner, to freezing and thawing, but there was no effect, as was to be expected.

Another portion of the filtered serum was not frozen, but used without any special treatment to inoculate 2 hogs, 1511 and 1512, each being given 22 c. c., and these animals were placed in a pen with hog 1513 as a check.

The diluted unfiltered blood was also used to test the effect of freezing on the virulence. One portion of the diluted unfiltered blood was frozen, as already described for the filtered blood. Hogs 1517 and 1518 were used for the inoculation of the frozen, unfiltered serum, and hog 1519 was placed in the pen as a check.

Hog 1465, the hog inoculated with 5 c. c. of the defibrinated blood of hog 1458, unfiltered, became sick 10 days after inoculation, grew steadily worse, and was etherized when in a very sick condition, 17 days after inoculation, and the blood used in another experiment, to be described later. There were no pronounced lesions seen at autopsy, no hemorrhages, and no ulcers in the intestines. Culture tubes inoculated from the liver and from the heart blood showed no growth of any kind; a tube inoculated from the spleen showed a contamination, but no *B. cholerae suis*.

Hog 1517, inoculated with unfiltered frozen serum, became sick in 7 days, and died 16 days after inoculation. *B. cholerae suis* was demonstrated in the heart blood, liver, and spleen.

Hog 1518, the other hog inoculated with unfiltered frozen serum, also showed symptoms of illness 7 days after inoculation, and was found dead next day. The autopsy showed in this case no reddening of the integument, but there were many punctiform ecchymoses in the subcutaneous fat. There were also many punctiform ecchymoses on the auricles and ventricles of the heart, in the kidneys, and in the intestines. In the intestines were also a number of ulcers. No cultures were taken.

Hog 1519, the uninoculated pen check on hogs 1517 and 1518, remained well and after 53 days was placed in the exposure pen, but 1 day later became so badly injured by other hogs in the pen that it had to be killed. There were no marked lesions of any kind except that the thyroid gland was greatly enlarged and reddened and showed on section a number of stellate ecchymoses. Cultures made from the heart, liver, and spleen failed to show the presence of *B. cholerae suis*.

Hog 1511, inoculated with the filtered unfrozen serum, became sick in 9 days and grew steadily worse, and was found dead 20 days after inoculation. The autopsy showed considerable reddening of the integument over the abdomen and thorax, and extensive thickening and adhesions in the pleuræ and in the pericardium. The

TABLE 30.

No. of hog.	Material injected.	Inoculation.		Exposure.		Autopsy.	Remarks.
		Date.	Result.	Date.	Result.		
1517	22 c. c. frozen unfiltered serum hog 1458.	1904. Oct. 3	1904 Sick Oct. 10; died Oct. 19	1904.	1904.	Hemorrhages.	<i>B. cholerae suis</i> present.
1518	do	do	Sick Oct. 10; died Oct. 11	Nov. 25	Killed Nov. 25.	do.	No cultures taken.
1519	Pen check on hogs 1517 and 1518.	do	Remained well.	Dec. 2	Remained well.	No lesions.	Do.
1520	22 c. c. frozen filtered serum hog 1458.	do	Sick Oct. 17; recovered	do	do	No lesions	No <i>B. cholerae suis</i> present. Cultures from organs did not grow.
1521	do	do	do	do	do	No lesions	<i>B. cholerae suis</i> present.
1522	Pen check on hogs 1520 and 1521.	do	Remained well.	do	Killed Dec. 17.	No marked lesions	<i>B. cholerae suis</i> not present.
1511	22 c. c. filtered serum hog 1458	do	Sick Oct. 12; died Oct. 23	Dec. 2	Remained well.	Ulcers	Do.
1512	do	do	Sick Oct. 12; recovered	Dec. 3	Dead Jan. 8, 1905	No marked lesions	
1513	Pen check on hogs 1511 and 1512	do	Remained well.	do	do	Slight lesions.	
1463	5 c. c. debrinated blood hog 1458.	Sept. 16	Sick Sept. 26; killed Oct. 3.				

lungs were hepatized and adherent to the thoracic wall by a dense, fibrinous exudate in the pleuræ. The heart was firmly bound to the pericardium by adhesions. The lesions, if any existed in the liver and spleen, were obscured by postmortem changes. The glands at the angles of the jaw were large and dark red. Cecum and ileocecal valve were studded with ulcers, varying in size from 1 mm. to 2 cm. in diameter. *B. cholerae suis* was obtained in cultures from the organs.

Hog 1512, the other hog inoculated with the Chamberland filtrate, unfrozen, became temporarily sick 9 days after inoculation, but after remaining sick for some time recovered, and was transferred to the exposure pen 62 days after inoculation, where the animal remained well.

Hog 1513, the uninoculated pen check on hogs 1511 and 1512, remained well and in 60 days was put in the exposure pen where it was found dead 1 month later, with no notable lesions of any sort. Culture tubes inoculated from the heart, liver, and spleen showed no growth.

Hog 1520, one of the hogs inoculated with filtered frozen serum, became somewhat sick 14 days after inoculation, but recovered and was placed in the exposure pen 60 days after inoculation and showed no symptoms of illness.

Hog 1521, the other hog inoculated at the same time as hog 1520 with the filtered frozen serum, also became sick 14 days after inoculation and recovered. This hog was also placed in the exposure pen 60 days after inoculation, and failed to show any symptoms of disease, but the animal was injured and had to be killed 15 days after exposure. The autopsy did not show any lesions worth noting, and culture tubes inoculated from the heart blood, from the liver, and from the spleen showed no growth.

Hog 1522, the uninoculated pen check on these 2, did not show any signs of illness, either in the pen with the inoculated animals or in the exposure pen where the animal was placed at the same time with hogs 1520 and 1521.

The results of the inoculations made with the blood of hog 1458 show that the unfiltered serum was infectious, and that successive freezing and thawing did not deprive the blood of virulence.

While all the hogs inoculated with the filtered serum, both frozen and unfrozen, became sick, only 1 of them died, 2 remained well on exposure to natural infection, and 1 was killed in the exposure pen.

The pen check on the hogs inoculated with the unfiltered frozen serum remained well, but was killed by the other hogs the day after being placed in the exposure pen. The pen check on the hogs inoculated with the filtered unfrozen serum remained well and died subsequently in the exposure pen, but showed no noticeable lesions. The pen check on the hogs inoculated with the filtered frozen serum remained well and did not sicken in the exposure pen.

The failure of the pen checks to contract the disease from the inoculated hogs is at variance with the results obtained in the great majority of our other experiments, and may be accounted for either by a low degree of infectiousness of the disease produced by the blood of hog 1458 or by an unusually high natural resisting power possessed by those animals. The fact that 1 remained well, and 1 died in 30 days without noticeable lesions when exposed to natural infection, inclines us to the latter view. As the remaining pen check was killed by other hogs 1 day after being placed in the exposure pen, this hog can not be taken into account.

EXPERIMENTS WITH BLOOD FROM HERD IV.

Advantage was taken of a spontaneous outbreak of hog cholera, which occurred in a herd of hogs which had been placed in quarantine at the Experiment Station of this Bureau in 1904, to get blood for the following filtration experiments—Nos. 18, 19, and 20:

FILTRATION EXPERIMENT No. 18.

Hog 1565, the animal from which the blood was obtained for this experiment, was born at the station in 1904, contracted hog cholera spontaneously, and when very sick, October 20, 1904, was etherized. The blood was drawn from the heart and defibrinated. The autopsy showed slight reddening of the skin in the thoracic region; glands at the angles of the jaw somewhat reddened; liver, spleen, and kidneys showed a number of punctiform ecchymoses; mucosa of the small intestine was reddened in places; cecum studded with typical small button ulcers. *B. cholerae suis* was obtained in cultures from the organs.

After the blood had stood in the refrigerator overnight, the serum was drawn off October 21, and was then diluted, in the usual proportions, with sterilized beef broth, 1 part of serum to 10 parts of beef broth, mixed with about 3 or 4 c. c. of a beef-broth culture of *B. prodigiosus*, and filtered through a Chamberland "F" cylinder. After the filtrate had been in the incubator for 3 days without clouding, a portion of it was used to inoculate 2 hogs. A portion of the serum was drawn off from the defibrinated blood, which had remained in the refrigerator meanwhile for 4 days, and this was diluted in the usual proportions—1 part of serum to 10 parts of beef broth—and used unfiltered to inoculate 2 hogs.

Hog 1573 was injected subcutaneously with 22 c. c. of the unfiltered diluted blood of hog 1565 and placed in the pen with hog 1574, which received the same, and with a pen check, hog 1575; seemed very sick 9 days after inoculation, and grew steadily worse until it was etherized and blood drawn from the heart 12 days after inoculation for another experiment. Autopsy showed lungs, liver, and kidneys to contain a number of punctiform ecchymoses. *B. cholerae suis* was obtained from the heart, liver, and spleen.

Hog 1574 was injected in the same manner as hog 1573; in 9 days seemed slightly sick; found dead 22 days after inoculation. Autopsy showed abdomen, thighs, and axillæ reddened; lymphatic glands at various locations enlarged and red; no ecchymoses in any of the organs; at base of ileocecal valve two small erosions. *B. cholerae suis* cultures were obtained from various organs.

Hog 1575 was not injected, but was placed in the pen with hogs 1573 and 1574 as a check; seemed slightly sick in 15 days, and 4 days later, when very sick and weak, was etherized and blood drawn from heart. Autopsy showed glands at the angles of the jaw enlarged and reddened; mesenteric glands greatly enlarged and red, those near lower portion of ileum had necrotic centers; punctiform ecchymoses in lungs, spleen, and kidneys. *B. cholerae suis* was present in the liver, spleen, and blood.

Hog 1570 was injected subcutaneously with 25 c. c. of the Chamberland filtrate and placed in a pen with hog 1571, which received the same, and with a pen check, hog 1572. Hog 1570 was slightly sick in 11 days, quite sick 4 days later, and was very sick 26 days after inoculation, when blood was drawn from the tail, defibrinated, and used to inject 2 hogs. Hog 1570 was found dead 42 days after inoculation. The autopsy showed inguinal glands edematous; those at angles of jaw sprinkled with a few small hemorrhagic spots; lungs edematous, with a few punctiform hemorrhages and several small areas of congestion. The spleen was enlarged, softened, and dark in color; kidneys showed in cortices, few small hemorrhagic spots; small intestine was very much congested; cecum and colon showed many erosions from 1 to 4 mm. in diameter. *B. cholerae suis* was obtained in cultures from the organs.

Hog 1571 was injected with the same material as hog 1570, and the clinical history is exactly the same in this case as with hog 1570, the animal dying 39 days after inoculation. The autopsy showed no hemorrhagic lesions, except a few punctiform ecchymoses in some of the lymph glands, and no ulcers in the intestines. The spleen was greatly enlarged. There was no growth in culture media inoculated from the heart, liver, and spleen.

Hog 1572 was not injected, but placed in pen as a check on hogs 1570 and 1571; seemed to be slightly sick in 12 days, and was found dead 14 days after being placed in the pen with hogs 1570 and 1571. Autopsy showed numerous ecchymoses in subcutaneous tissues over the abdomen and chest; also ecchymoses of various sizes from 2.5 cm. in diameter and under, in the abdominal muscles and in the muscles of the legs. Inguinal glands and those at angles of the jaw, very dark red; quite a large blood clot covering an area several inches in diameter was found in the neighborhood of ensiform cartilage; mediastinal and pelvic glands and mesenteric glands very dark red; entire external surface of the heart covered with punctiform ecchymoses; numerous diffuse hemorrhages on the surface of the peritoneum; spleen enlarged and dark; serous surface of the stomach showed a number of punctiform ecchymoses, especially abundant on cardiac extremity; kidneys showed a few punctiform ecchymoses, and the pelvis of the right kidney was filled with a dark, fibrous clot; entire serous surface of the bladder was covered by ecchymoses; the mucous surface showed the same; the intestines, both large and small, showed on the serous surface, as well as on the mucous surface, a very large number of ecchymoses; culture media inoculated from the heart, liver, and spleen, showed no growth whatever.

Hog 1597, injected with 5 c. c. of defibrinated blood of hog 1570 (*intra vitam*) became slightly sick in 13 days, and grew steadily worse until the animal was killed 49 days after inoculation. The autopsy showed no important lesions except at the ileocecal valve, where there was an erosion covered with an adherent yellowish mass. Culture tubes inoculated from the lungs, liver, and spleen showed no growth.

Hog 1598 was injected the same as hog 1597, became slightly sick in 13 days, and remained slightly sick until 20 days later, then grew gradually worse until the animal was found dead 39 days after inoculation. There was marked reddening of the skin over the abdomen, thighs, and in the axillæ. The mesenteric, retroperitoneal, and lumbar glands were dark red, and a few erosions of the mucosa of the ileum and cecum in neighborhood of the ileocecal valve were found. *B. cholerae suis* was obtained in cultures from the heart, liver, and spleen.

Hog 1599 was not injected but placed in pen with hogs 1597 and 1598 as a check, became sick in 21 days, and was found dead 49 days after being placed in the pen with hogs 1597 and 1598. The only important lesions found in this case were consolida-

TABLE 31.

No. of hog.	Material injected.	Inoculation.		Autopsy.	Remarks.
		Date.	Result.		
1573	22 c. c. unfiltered serum hog 1565	1904 Oct. 24	1904 Sick Nov. 2; killed Nov. 5	Hemorrhages	<i>B. cholerae suis</i> present.
1574	do	do	Sick Nov. 2; died Nov. 15	Hemorrhages and ulcers	Do.
1575	Pen check on hogs 1573 and 1574	do	Sick Nov. 3; killed Nov. 12	Hemorrhages and ulcers	Do.
1576	25 c. c. filtered serum hog 1565	Oct. 24	Sick Nov. 4; died Dec. 3	Hemorrhages and ulcers	Do.
1577	do	do	Sick Nov. 5; died Dec. 7	Glands hemorrhagic	No growth from organs.
1578	do	do	Sick Nov. 5; died Nov. 7	Hemorrhages	Do.
1579	Pen check on hogs 1570 and 1571	(a) Nov. 16	Sick Nov. 24; killed Jan. 4, 1905	Ulcers	Do.
1580	Defibrinated blood hog 1570, intra vitam	do	Sick Nov. 24; dead Dec. 29	do	<i>B. cholerae suis</i> present.
1581	do	(b)	Sick Nov. 24; dead Dec. 29	Lung lesions.	No growth from organs.
1582	do	do	Sick Dec. 7; died Jan. 4, 1905	do	do
1583	Pen check on hogs 1587 and 1598	do	do	do	do

^a Exposed October 24.^b Exposed November 16.

tion of the cephalic lobes in both lungs with necrotic foci throughout. Culture tubes inoculated from the liver, heart blood, and spleen showed no growth of any kind.

It will be observed that in this experiment the animals inoculated with the unfiltered diluted serum became sick somewhat earlier than those inoculated with the filtered serum, and that the uninoculated check animal in the pen with the hogs inoculated with the filtered serum showed signs of sickness almost as soon as the inoculated hogs. This, however, is no cause for surprise, because it is probable that the inoculated animals were really sick before symptoms were noticed, and were consequently capable of conveying the contagion before any symptoms were sufficiently pronounced to be observed. The possibility of such an occurrence is suggested in the two tail-blood experiments where the blood was found to be infectious 2 days after inoculation, although the hogs at that time showed no symptoms whatever of sickness. The result of the inoculation of hogs 1597 and 1598 with the blood of hog 1570, while the latter was sick, demonstrates the infectiousness of the blood of animals inoculated with the filtered virulent blood, and, furthermore, the fact that the check hog 1599 contracted the disease from association with hogs 1597 and 1598 shows that the disease was contagious.

FILTRATION EXPERIMENT No. 19.

Hog 1573 was injected October 24, 1904, as already explained in Filtration Experiment No. 18. The blood was drawn from the heart, defibrinated, and placed in the refrigerator, and 2 days later 50 c. c. of serum was drawn off and diluted with 500 c. c. of sterilized beef broth. As usual, a thick suspension of *B. prodigiosus* in about 4 or 5 c. c.

of beef broth was added to the mixture. One portion of about 150 c. c. of the diluted serum was reserved before the addition of *B. prodigiosus* and placed in the refrigerator until the next day, when it was used for the injection of 2 hogs. The remainder of the diluted serum was filtered through a Chamberland "F" cylinder.

About 100 c. c. of the filtrate was removed with a sterile pipette and placed in the refrigerator overnight. The rest of the filtrate, about 200 c. c., was placed in the incubator. The portion of the filtrate which had been placed in the refrigerator was used the next day for the inoculation of 2 rabbits and 2 hogs. The portion placed in the incubator became cloudy, but this was evidently due to contamination occurring at the time when the portion of the filtrate which was used for the inoculation of the rabbits and hogs was drawn off, for careful culture tests and examination with the microscope failed to show any organism resembling *B. cholerae suis* or *B. prodigiosus*. The 2 rabbits inoculated with 22 c. c. each of the filtered serum remained perfectly well.

Hog 1584 was injected with 22 c. c. of diluted unfiltered serum of hog 1573, showed slight signs of sickness 9 days later, grew rapidly worse, and was found dead 13 days after inoculation. Autopsy revealed numerous punctiform ecchymoses in the lungs, heart, liver, spleen, kidneys, mucosa of the stomach, mucosa of the small intestine, mucosa of the cecum and colon, many small and a few large ulcers in the colon, many ecchymoses in the rectum. No cultures were taken in this case.

Hog 1585 was injected with the same material as hog 1584, became sick 9 days later, grew rapidly worse, and died 14 days after inoculation. Autopsy revealed the glands at the angles of the jaw much enlarged and reddened, heart showed numerous hemorrhages, kidneys showed a number of punctiform ecchymoses on the surface and throughout the substance of the organs, abdominal lymph glands enlarged and reddened. *B. cholerae suis* was obtained in cultures from the heart blood, liver, and spleen.

Hog 1586 was not injected, but was placed in the pen with hogs 1584 and 1585 as a check; slightly sick 18 days later; grew rapidly worse, and was found dead 26 days after being placed in experiment pen. Ventral surface of the body showed numerous reddened patches, a large number of punctiform hemorrhages in the liver, kidneys, and mucosa of the small intestine; numerous small and a few large button ulcers in the cecum and colon. No cultures were taken in this case.

Hog 1582 was injected subcutaneously with 22 c. c. of Chamberland "F" filtrate from serum of hog 1573; was slightly sick 13 days after inoculation; symptoms gradually increased in severity, but the animal, although desperately sick, did not die until 49 days after inoculation. Autopsy revealed lymphatic glands generally enlarged and hemorrhagic; peribronchial and mediastinal glands also enlarged and hemorrhagic; spleen greatly enlarged, soft, and friable; a few punctiform ecchymoses in the kidneys; a few erosions in cecum and colon. Pure culture of *B. coli communis* was obtained from the heart blood.

Hog 1583 was injected with the same material as hog 1582; became slightly sick 8 days after inoculation, grew steadily worse, and was found dead 40 days after inoculation. Autopsy revealed extensive reddening of the integument over the abdomen, groins, and throat. Inguinal glands and those at the angles of the jaw enlarged but not hemorrhagic; spleen greatly enlarged and friable; punctiform ecchymoses were found in the mucosa of duodenum; superficial erosions in the cecum and enlargement and reddening of the abdominal lymphatic glands. *B. cholerae suis* was obtained in cultures from the heart blood, liver, and spleen.

Hog 1557 was not injected, but placed in the pen with hogs 1582 and 1583 as a check; became slightly sick 18 days later, and grew steadily worse until death, 27 days after being placed in the pen. Autopsy revealed several areas of congestion in the lungs; congestion of the spleen; congestion of the mucosa of the small intestine; many "button" ulcers in the cecum and colon. No cultures were taken in this case.

TABLE 32.

No. of hog.	Material injected.	Inoculation.		Autopsy.	Remarks.
		Date.	Result.		
1584	22 c. c. unfiltered serum hog 1573.	1904. Nov. 8	1904. Sick Nov. 17; dead Nov. 21.	Hemorrhages.	No cultures taken.
1585	do	do	Sick Nov. 17; dead Nov. 22.	do	<i>B. cholerae suis</i> present.
1586	Pen check on hogs 1584 and 1585.	(a)	Sick Nov. 26; dead Dec. 4.	Hemorrhages and ulcers.	No cultures taken.
1582	22 c. c. filtered serum hog 1573.	Nov. 8	Sick Nov. 21; died Dec. 27.	Hemorrhages.	<i>B. coli communis</i> from heart blood.
1583	do	do	Sick Nov. 16; died Dec. 18.	Hemorrhages in intestines.	<i>B. cholerae suis</i> present.
1557	Pen check on hogs 1582 and 1583.	(a)	Sick Nov. 26; died Dec. 5.	Intestinal ulcers.	No cultures taken.

^a Exposed November 8, 1904.

The results of this experiment show that the filtered blood produced fatal disease in hogs inoculated with it, and that the uninoculated pen check became sick from association with these hogs, showing the first symptoms 5 days after the disease appeared in the first of the inoculated animals. The lesions in the inoculated animals were those of an acute hemorrhagic septicemia. The lesions in the check animal were those of the chronic intestinal type.

The rabbits inoculated with the same amount of filtered serum as hogs 1582 and 1583 showed no signs of sickness.

The hogs inoculated with the unfiltered serum developed symptoms of sickness approximately at the same time as those inoculated with filtered serum, but died more promptly than these, and the pen check on these animals also contracted disease and died.

FILTRATION EXPERIMENT No. 20.

Hog 1575, the animal furnishing blood for this experiment, is described on page 80. The blood was drawn, defibrinated, and, after it had stood in the refrigerator for 4 days, the serum was drawn off, mixed with sterilized beef broth in the proportion of 1 to 10, and to this mixture was added, as usual, 4 or 5 c. c. of a beef broth suspension of *B. prodigiosus*, and the whole was then filtered through a Chamberland "F" cylinder. The serum in this case contained such a large number of red blood cells that filtration was much retarded. Only about 130 c. c. of filtrate was obtained, all of which was used for the inoculation of 4 hogs and 2 rabbits. One of the rabbits received subcutaneously 17 c. c. of the filtrate and the other 22 c. c. Both remained perfectly well. Two of the hogs will be described at some future date in connection with a different set of experiments, since the blood was subjected to certain treatment and does not properly belong in the present set of experiments.

Hog 1609 was injected subcutaneously with 22 c. c. of diluted unfiltered blood of hog 1575; became sick in 9 days, grew rapidly worse, and was found dead 14 days after inoculation. Autopsy showed punctiform ecchymoses in the heart, kidneys, and mucosa of the bladder; congestion of the spleen and mucosa of the stomach. A mass of clotted blood was firmly adherent to the mucosa of the ileum and the

ileocecal valve. Cultures from the heart blood, liver, and spleen showed the presence of *B. cholerae suis*.

Hog 1610 was injected subcutaneously, the same as hog 1609; became sick in 9 days, grew rapidly worse, and was found dead 14 days after inoculation. Autopsy showed considerable reddening of the integument over the abdomen; a few punctiform ecchymoses in the lungs and kidneys; enlargement of the spleen; enlargement and congestion of the lymphatic glands generally. *B. cholerae suis* obtained in cultures from the heart blood, liver, and spleen.

Hog 1611 was not injected, but placed in pen with hogs 1609 and 1610 as a check; was slightly sick in 15 days, and grew gradually worse until death, 11 days later. Autopsy showed punctiform ecchymoses in the heart and kidneys; spleen somewhat enlarged. The lymphatic glands generally were large and congested. The skin over the entire abdomen reddened. Cultures of *B. cholerae suis* were obtained from the organs.

Hog 1603 was injected subcutaneously with Chamberland "F" filtrate from the blood of hog 1575, became very sick in 12 days, and grew steadily worse and was killed 22 days after inoculation. Autopsy revealed a number of punctiform ecchymoses in the heart; congestion of the liver; congestion and ecchymoses in the spleen; abdominal lymph glands large and dark; yellow exudate firmly adherent to mucosa of small intestine; numerous punctiform ecchymoses in colon; also numerous ecchymoses and ulcers in the cecum. The method of taking cultures in this case differed from that usually employed, in that 4 flasks containing 100 c. c. each of sterilized beef broth were inoculated with 10 c. c. each of blood drawn from the tail previous to killing the animal. These flasks were placed in the incubator and 3 of them gradually became clouded. One of them remained perfectly clear. Guinea pigs and rabbits were inoculated with $\frac{1}{2}$ to $\frac{1}{4}$ c. c. from each one of the clouded flasks, and plate cultures were also made from the flasks. The plate cultures failed to reveal any organism resembling *B. cholerae suis*. None of the rabbits were affected injuriously and are alive and well to date, January 26, 1905. The guinea pigs died on the eighth, tenth, and thirteenth days, respectively, after inoculation. Culture media inoculated from the organs of these guinea pigs failed to show any growth from 2 of them, but culture media inoculated from the tissues of the guinea pig which died 13 days after inoculation showed the presence of *B. cholerae suis*. The length of time elapsing between the inoculation of this guinea pig and death, taken together with the fact that the rabbit inoculated from the same flask remained well, makes it seem probable that infection was accidental.

Hog 1604 was injected the same as hog 1603, became slightly sick in 16 days, and remained sick for about 5 or 6 days, then improved, but became sick again, and finally died 60 days after inoculation. Autopsy revealed extensive ulceration of the integument over the abdomen, throat, and inside the thighs; the lymphatic gland at the angle of the jaw on the right side was large and red; an old erosion, to which was adherent a reddish crust, was found at the base of the ileocecal valve; spleen was enlarged and dark; the glands at the brim of the pelvis were enlarged and reddened. Culture media inoculated from the heart, liver, and spleen showed no growth of any kind.

Hog 1605, uninoculated, was placed in the pen with hogs 1603 and 1604 as a check; became sick in 27 days, and grew gradually worse until death, 68 days after inoculation. The autopsy showed reddening of the lymph glands and numerous diffuse, irregular erosions, but no distinct button ulcers in the intestines. Cultures from the heart blood, liver, and spleen showed the presence of *B. cholerae suis*.

TABLE 33.

No. of hog.	Material injected.	Inoculation.		Autopsy.	Remarks.
		Date.	Result.		
1609	22 c. c. unfiltered serum hog 1575.	1904. Nov. 17	1904. Sick Nov. 26; dead Dec. 1.	Hemorrhages.	<i>B. cholerae suis</i> present.
1610	do	do	do	do	Do.
1611	Pen check on hogs 1609 and 1610.	(^a)	Sick Dec. 2; dead Dec. 13.	do	Do.
1603	22 c. c. filtered serum hog 1575.	Nov. 17	Sick Nov. 29; killed Dec. 9.	do	<i>B. cholerae suis</i> not present in organs.
1604	do	do	Sick Dec. 3; died Jan. 16, 1905.	Hemorrhages; erosions.	No growth from organs.
1605	Pen check on hogs 1603 and 1604.	(^a)	Sick Dec. 14; died Jan. 24, 1905.	Erosions in cecum.	<i>B. cholerae suis</i> present.

^a Exposed November 17.

It will be noticed that in this experiment the hogs inoculated with the filtered blood both became sick after a period of incubation of 12 and 16 days, respectively, and that the autopsy showed hemorrhagic lesions in both cases. The uninoculated check became sick, or rather symptoms of illness were noticed, some time after the inoculated animals, and also showed hemorrhages and, in addition, erosions in the intestines. *B. cholerae suis* was present in one of the inoculated animals, but could not be detected in the other, while it was found in the check.

One rabbit inoculated with a dose of the filtered serum equal to that used for the hogs, and another receiving almost as much, failed to show any signs of sickness.

The hogs inoculated with the unfiltered blood, as well as the check, all behaved as usual in such cases. The symptoms showed in these animals 3 days before they were noticed in the hogs inoculated with the filtered serum, and the disease ran a more acute course.

EXPERIMENT WITH BLOOD FROM HERD V.

The disease in this herd, located in southwestern Iowa, appeared about July 8, 1904, the herd numbering at that time 103 hogs—16 of them old sows and 87 pigs. An examination of the herd on July 28, 1904, when the animal was secured for the experiment described below, showed that 2 of the sows and 28 of the pigs had died; the disease at that time was characterized by the usual hog-cholera symptoms; that is, sore eyes, cough, weakness in hind legs, and diarrhea, the latter being a prominent symptom. A final report on this herd, dated November 2, 1904, states that of the 16 old sows 14 had died; the remaining 2 were sick but recovered; and that of the 87 pigs 84 died and 3 were never sick.

FILTRATION EXPERIMENT No. 21.

A hog was selected from herd V, the animal being in a very sick condition at the time. This hog, designated hog No. 1 from herd V, was etherized in order to obtain blood for the experiment described below. The autopsy on this animal showed enlarged inguinal glands; partial consolidation of cephalic lobes of both lungs; spleen considerably enlarged, very dark, and soft; small punctate hemorrhages in the kidneys; a number of large circular ulcers from 1 to 1.5 cm. in diameter in the cecum. Cultures from heart blood and spleen failed to show *B. cholerae suis*.

The heart blood of hog 1 from herd V was drawn by means of a sterile canula, defibrinated, filtered through absorbent cotton, and placed on ice overnight. At the end of 24 hours the serum was drawn off with a sterile pipette, but, owing to the fact that the red blood corpuscles failed to settle out well, the serum obtained was quite red. To 35 c. c. of the serum was then added 350 c. c. of neutral beef broth

and 3 c. c. of a suspension of *B. prodigiosus* in sterile salt solution, and the mixture was then filtered through a Chamberland "F" cylinder. Owing to the admixture of red blood cells, the filtration was much retarded and was discontinued at the end of an hour, when a little over 100 c. c. had passed through the filter.

Portions of the diluted serum, before and after filtration, were drawn off by means of sterile pipettes and set aside for the inoculation of experiment animals. The portion of diluted filtered serum which remained was set to one side and showed clouding at the end of 48 hours; agar plates were then made from this filtrate and showed (1) a long, rather slender bacillus, and (2) a large thick bacillus, but no colonies of *B. cholerae suis* nor *B. prodigiosus*.

The following is a list of the animals inoculated, with brief clinical notes and autopsy records in each case:

Hog 184 was injected subcutaneously with 25 c. c. of diluted unfiltered serum of hog 1 from herd V; showed first symptoms of sickness on the sixth day; was very sick, and exhibited diarrhea on the tenth day, and died on the eleventh day after inoculation. Autopsy showed considerable reddening of skin over throat; inguinal glands and lymphatics generally dark and swollen; numerous small, circumscribed areas of congestion in lungs; spleen enlarged, very dark, and soft; kidneys congested and studded with small punctate hemorrhages; numerous small hemorrhagic splotches on serous surface of small intestine, and mucosa reddened; mucosa of large intestine showed hemorrhagic splotches, which had the appearance of beginning ulceration; mucosa over pyloric end of stomach intensely reddened; several hemorrhagic splotches on left ventricle and numerous small hemorrhagic splotches on both auricular appendages. Cultures from heart blood and spleen showed *B. cholerae suis*.

Hog 185 was injected subcutaneously, the same as hog 184; was first noticed sick on the sixth day; was very sick, and showed diarrhea on the sixteenth day, and died on the nineteenth day after inoculation. Autopsy showed inguinal glands and lymphatics generally enlarged and dark; lungs thickly studded with small patches of congestion; spleen very much enlarged, very dark, and soft; kidneys congested, with a small number of punctate hemorrhages; a few small hemorrhagic splotches on left auricular appendage; mucosa over pyloric end of stomach intensely reddened; cecum showed numerous shallow ulcers, covered with a thick, tenacious, yellow exudate; mucosa of large intestine very red and edematous. Agar cultures from heart blood and spleen showed *B. cholerae suis*.

Hog 181 was injected subcutaneously with 25 c. c. of diluted filtered serum of hog 1 from herd V and placed in a pen with hogs 182 and 183; showed first symptoms of sickness on eighth day, was very sick and exhibited diarrhea on eleventh day, and died on the twelfth day after inoculation. Autopsy showed inguinal glands enlarged and somewhat dark; small patches of congestion in lungs; several hemorrhagic splotches on ventricles, and numerous small hemorrhagic splotches on auricular appendages; spleen enlarged, very dark, and soft; kidneys congested and thickly studded with small punctate hemorrhages; mucosa over pyloric end of stomach intensely reddened; numerous small hemorrhagic splotches on serous surface of small intestine, mucosa reddened; mucosa of cecum and large intestine slightly reddened. Cultures from heart blood and spleen showed *B. cholerae suis*.

Hog 182 was injected the same as hog 181; showed first symptoms of sickness on eighth day, became gradually worse, and died 19 days after inoculation. Autopsy showed inguinal glands enlarged and slightly hyperemic; spleen somewhat enlarged, dark and quite soft; kidneys congested and studded with small punctate hemorrhages; a few hemorrhagic patches on left ventricle; mucosa over pyloric end of stomach slightly reddened; small, early ulcerations in mucosa of cecum near ileocecal valve; large intestine showed extensive ulceration, the mucosa having the appearance of one large continuous ulcer. Cultures from heart blood and spleen showed *B. cholerae suis*.

Hog 183 was not inoculated but placed in pen with hogs 181 and 182 as a check; showed first symptoms of sickness on the fifteenth day, was very sick on the nineteenth day, and died on the twentieth day after being placed in the pen. Autopsy

showed inguinal glands and lymphatics generally dark and congested; a few scattered areas of congestion in the lungs; a few small hemorrhages on the right auricle; spleen somewhat enlarged, very dark and soft; kidneys congested and thickly studded with small punctate hemorrhages; mucosa over pyloric end of stomach intensely reddened; mucosa of cecum showed a few hemorrhagic patches, and the mucosa of large intestine was reddened. Cultures from the heart blood and spleen showed *B. cholerae suis*.

TABLE 34.

No. of hog.	Material injected.	Inoculation.		Autopsy.	Remarks.
		Date.	Result.		
184	25 c. c. unfiltered serum hog 1, herd V.	1904. July 27	1904. Sick Aug. 2; died Aug. 7.	Hemorrhages; beginning ulceration of cecum and colon.	<i>B. cholerae suis</i> present.
185	do.....	do.....	Sick Aug. 2; died Aug. 15.	Hemorrhages and ulcers.	Do.
181	25 c. c. filtered serum hog 1, herd V.	do.....	Sick Aug. 4; died Aug. 8.	Hemorrhages.	Do.
182	do.....	do.....	Sick Aug. 4; died Aug. 15.	Hemorrhages and ulcers.	Do.
183	Check, exposed to hogs 181 and 182.	(a)	Sick Aug. 11; died Aug. 16.	Hemorrhages.	Do.

^a Exposed July 27.

In this experiment 2 hogs were inoculated with the unfiltered serum and 2 with the filtered serum.

The 2 hogs, 184 and 185, inoculated with the unfiltered serum died with hemorrhagic lesions and intestinal ulceration on the eleventh and nineteenth days, respectively, after inoculation.

Hogs 181 and 182, inoculated with the filtered serum, died with hemorrhagic lesions on the twelfth and nineteenth days, respectively, after inoculation, and one of these animals, hog 182, showed ulceration of the large intestine. Hog 183 contracted the disease through exposure to hogs 181 and 182, and died with hemorrhagic lesions 20 days after being placed in the experiment pen with the filtered-serum animals.

B. cholerae suis was recovered in cultures from all of the animals used in this experiment.

It will be noticed that the filtrate in this experiment showed clouding at the end of 48 hours, but agar plates made from this filtrate after it became clouded failed to show *B. cholerae suis* or *B. prodigiosus*, and it is evident that the clouding was due to contamination by air organisms when the flask was opened to draw off the portion used in the inoculation of the experiment animals, and was not due to the passage of organisms through the filter.

EXPERIMENT WITH BLOOD FROM HERD VI.

The animal furnishing blood for this experiment was secured from an infected herd in Iowa. At the time the disease appeared, August 19, 1904, this herd numbered 7 old sows and 70 pigs, and on August 20,

when the animal was secured for filtration experiment, 4 pigs had died and 16 pigs and one old sow were sick. A later report, dated November 2, 1904, gives the total loss as 1 old sow and 61 pigs. Previous outbreaks of hog cholera occurred on the farm where this herd was kept in 1887, 1899, and in December, 1903.

At the time the hog was secured for the filtration experiment the animals were in bad condition, many of them very thin, dull, and drowsy, with swollen ears, and most of them very lousy. The affected animals exhibited marked weakness, with staggering gait, some cough, and profuse diarrhea. Two hogs were selected for postmortem examination as follows:

Hog 1: This animal was in a very weak condition, could scarcely rise, was scouring freely, and purple spots could be seen on abdomen. The autopsy revealed inguinal glands and lymphatics generally red and swollen; a few scattered hemorrhages in lungs; spleen enlarged, dark, and very soft; numerous petechiæ in kidneys; small hemorrhages apparent on both the serous and mucous surfaces, and large yellowish ulcers in the large intestine, especially numerous in the cecum.

Hog 2: This animal, which was in a very weak condition, but not quite as sick as hog 1, was etherized, and the heart blood, drawn by means of a sterile canula, was used in the experiment described below. The autopsy showed red and swollen inguinal glands; scattered, circumscribed areas of congestion in the lungs; hemorrhages on auricular appendages and left ventricle of heart; spleen slightly enlarged and dark; kidneys congested and thickly studded with punctate hemorrhages; numerous typical button ulcers in cecum and colon, and numerous adhesions between the colon and rectum and between the different folds of the large intestine; mesenteric and lumbar glands dark red, almost black. Agar cultures from heart blood showed *B. cholerae suis*.

FILTRATION EXPERIMENT NO. 21.

The defibrinated heart blood of hog 2, herd VI, was filtered through absorbent cotton and placed on ice overnight. After 21 hours, when the red blood corpuscles had settled out, the serum was drawn off with a sterile pipette. To 30 c. c. of the clear serum, free from red blood corpuscles, was added 300 c. c. of sterile beef broth, and 3 c. c. of a suspension of *B. prodigiosus* in bouillon was also added, and the whole passed through a Chamberland "F" cylinder.

A portion of the diluted serum, before filtration and before the addition of *B. prodigiosus*, was used for the inoculation of a hog. The clear filtrate was set away in the dark and allowed to stand at room temperature (about 25° C.) for 3 days, at the end of which time it remained perfectly clear and was then used for the inoculation of experiment animals.

In making the inoculations the following procedure was adopted: Hog 213 was first inoculated subcutaneously on the inside of the thigh with 25 c. c. of sterile bouillon. Hogs 211 and 212 were then inoculated in the same manner with 25 c. c. each of diluted filtered serum, using the same syringe (without subsequent sterilization) as was used for hog 213. These 3 animals were placed in one pen. The inoculation of the pen check with sterile bouillon was intended simply as a check on the sterilization of the syringe. A fourth animal, hog 215, received 25 c. c. of the diluted unfiltered serum and was placed in a separate pen.

TABLE 35.

No. of hog.	Material injected.	Inoculation.		Exposed.		Autopsy.	Remarks.
		Date.	Result.	Date.	Result.		
209	25 c. c. unfiltered serum hog 2, herd VI	1904, Aug. 24	Sick Sept. 4; died Sept. 10.	1904.	1904.	Hemorrhages and ulcers	<i>B. cholerae suis</i> present.
211	25 c. c. filtered serum hog 2, herd VI	Aug. 27	Sick Sept. 7; died Sept. 16.	Sept. 29	Remained well.	do	Do
212	do	do	Remained well.	do	do	do	do
213	25 c. c. sterilized bouillon, check, exposed to hogs 211 and 212.	do	do	do	do	do	do

The following is a list of the animals inoculated, with brief clinical notes and autopsy records of each:

Hog 209 was injected subcutaneously with 25 c. c. of diluted unfiltered serum of hog 2 from herd VI; showed first symptoms of sickness after 11 days; became gradually worse, developed diarrhea, and died 17 days after receiving the injection. Autopsy showed slight reddening of integument in axillæ and groins; inguinal glands red and swollen; almost complete consolidation of both lungs, with adhesions to the chest wall and diaphragm; liver dark and much congested; spleen slightly enlarged, dark and soft, with large dark, slightly raised smooth areas on the surface; kidneys congested, with scattered punctate hemorrhages; mucosa of stomach much reddened toward pyloric end; large, shallow ulcerations in cecum; mesenteric glands enlarged and reddened. Agar cultures from heart blood and spleen showed pure growths of *B. cholerae suis*.

Hog 211 was injected subcutaneously with 25 c. c. of diluted filtered serum of hog 2, herd VI; showed first symptoms of sickness after 11 days; became gradually worse, and died 20 days after the injection. Autopsy showed enlarged and reddened inguinal glands; small, hemorrhagic points on auricular appendages of heart; large areas of congestion and scattered hemorrhagic points in the lungs; spleen much enlarged, dark, and soft; kidneys large and soft, with small, punctate hemorrhages; mucosa of stomach intensely congested and covered with a yellow, necrotic exudate; mucosa of cecum and colon thickly studded with small, punctate hemorrhages and beginning ulceration in the cecum; lumbar glands enlarged and dark. Agar cultures from heart blood and spleen showed pure growths of *B. cholerae suis*.

Hog 212 was injected subcutaneously with 25 c. c. of diluted filtered serum of hog 2 from herd VI. This animal was kept under careful observation for 33 days and remained well, except for slight loss in weight. The animal was then kept in the exposure pen for 29 days and remained well.

Hog 213 was injected subcutaneously with 25 c. c. of sterile bouillon and exposed in pen with hogs 211 and 212. This animal was kept under observation for 33 days and remained well. It was then placed in the exposure pen, where it was kept for 29 days and remained well.

In summarizing the results obtained in this experiment we find that hog 209, the animal inoculated with unfiltered serum, died with hemorrhagic lesions and ulcers in the cecum on the seventeenth day after inoculation.

Hog 211, one of the animals inoculated with filtered serum, died with hemorrhagic lesions and beginning ulceration in the cecum on the twentieth day after inoculation. Hog 212, the other animal inoculated with filtered serum, showed no ill effects from the inoculation, other than a slight loss in weight, and was

subsequently immune when placed in the exposure pen. Hog 213, the pen check on the 2 preceding animals, remained well and was likewise immune when placed in the exposure pen. It is impossible to say with certainty whether the immunity from natural infection shown by hogs 212 and 213 was natural or acquired.

The filtrate used in this experiment was kept for 3 days at room temperature, about 25° C., a temperature at which *B. cholerae suis* develops readily, and remained perfectly clear. It is evident, therefore, that the material used for the injection of hogs 211 and 212 was entirely free from *B. cholerae suis*.

EXPERIMENT WITH BLOOD FROM HERD VII.

The animal furnishing blood for this experiment was obtained from a herd in Iowa. Disease appeared in this herd about the 1st of August, 1904, at which time the herd numbered 14 old hogs and 87 pigs. Of the old hogs, 8 died, the others were not affected; of the pigs, 83 died, and the remaining 4 were sick, but recovered. In this herd constipation was a prominent symptom, and reddening of the skin was present in many of the cases. The farm on which this herd was located was visited by an epidemic of hog cholera 5 years previous to the present outbreak.

On August 9, 1904, when the disease seemed at its worst, 2 hogs were selected for postmortem examination, as follows:

Hog 1: This animal, which weighed about 60 pounds, had shown some slight diarrhea. The autopsy showed considerable reddening over abdomen and flanks; ears swollen and dark purple in color; inguinal glands and lymphatics generally red and swollen; lungs showed small hemorrhagic patches and larger areas of congestion; spleen enlarged and dark; a few small punctuate hemorrhages in the kidneys; a few small, round, dark ulcers in the mucosa of cecum and colon. No cultures were made.

Hog 2: This animal, which weighed about 50 pounds, was etherized on August 9, 1904, and the heart blood, drawn by means of a sterile canula, was used in the experiment described below. The autopsy on this animal showed intense reddening of skin over throat and upper part of thorax; inguinal glands enlarged and firm; a few small, scattered, circumscribed areas of congestion in the lungs; small hemorrhagic splotches on both auricular appendages; spleen enlarged, dark, and soft; a few punctate hemorrhages in the kidneys; mucosa of small intestine slightly reddened; mucosa of cecum showed several large hemorrhagic patches and beginning ulceration near the ileocecal valve. Cultures from heart blood and spleen showed pure growths of *B. cholerae suis*.

FILTRATION EXPERIMENT No. 22.

The defibrinated heart blood of hog 2, herd VII, was filtered through absorbent cotton and allowed to stand on ice overnight. At the end of 19 hours the clear serum was drawn off with a sterile pipette. To 50 c. c. of the clear serum, free from red blood corpuscles, was added 500 c. c. of neutral beef broth and 3 c. c. of a 48-hour bouillon culture of *B. prodigiosus*, and the whole passed through a Chamberland "F" cylinder. The filtrate was collected in two portions of 250 c. c., each in separate side-arm flasks. About 50 c. c. of the diluted serum remained in the filter when the filtration was discontinued.

A portion of the diluted serum, before filtration and before the addition of *B. prodigiosus*, was used for the inoculation of a hog. Cultures from the diluted serum previous to filtration showed *B. cholerae suis*. From the first portion of the filtrate a sufficient quantity was drawn off with a sterile pipette for the inoculation of hogs, and the two filtrates were then set aside in the dark.

At the end of 48 hours the first portion showed clouding, whereas the second portion remained clear at the end of a week, from which fact it would seem quite certain that the clouding in the first instance was due to contamination by air organisms at the time the portion was drawn off for the inoculation of the experiment animals, and this supposition is further borne out by the microscopic examination of the portion which became clouded, for this failed to show *B. cholerae suis*.

The following is a list of animals inoculated, with brief clinical notes and autopsy records in each case:

Hog 176 was injected subcutaneously with 25 c. c. of the diluted unfiltered serum from hog 2, and was kept under careful daily observation for 21 days. The animal showed slight indisposition on the seventh, eighth, and ninth days after inoculation, but recovered and was placed in the exposure pen 21 days after inoculation, where it remained for 36 days without exhibiting any symptoms of disease.

Hog 195 was injected subcutaneously with 25 c. c. of the diluted filtered serum from hog 2 and placed in a pen with hogs 196 and 197; was first noticed sick 9 days after inoculation and was quite sick for 12 days. The animal then began to improve and made a good recovery, but failed to recover loss in flesh; was placed in the exposure pen 44 days after inoculation. It was kept in the exposure pen for 35 days and remained well.

Hog 196 was injected in the same manner as hog 195. The record of this animal is exactly the same as that of hog 195; was first noticed sick 9 days after inoculation, and was quite sick for 12 days. It then began to improve and made good recovery, but failed to recover loss in flesh. Was placed in exposure pen, where it was kept for 35 days and remained well.

Hog 197 was injected subcutaneously with 25 c. c. of sterile bouillon and exposed in pen with hogs 195 and 196. This animal remained perfectly well, was placed in exposure pen after 44 days and died in 17 days. Autopsy showed hemorrhagic lesions.

TABLE 36.

No. of hog.	Material injected.	Inoculation.		Exposed.		Remarks.
		Date.	Result.	Date.	Result.	
176	25 c. c. unfiltered serum hog 2, herd VII.	1904. Aug. 10	1904. Slightly sick Aug. 17 to 19; recovered.	1904. Sept. 1	Remained well.	Kept in exposure pen from Sept. 1 to Oct. 7 (37 days); remained well.
195	25 c. c. filtered serum hog 2, herd VII.	do....	Sick Aug. 19 to Sept. 16 recovered.	Sept. 23	do.....	Kept in exposure pen from Sept. 23 to Oct. 28 (35 days); remained well.
196	do.....	do....	do.....	do....	do.....	Do.
197	25 c. c. sterilized bouillon; check, exposed to hogs 195 and 196.	do....	Remained well.	do....	Died Oct. 10.	Autopsy revealed hemorrhages and ulcers.

In summarizing the results obtained in this experiment we find that hog 176, inoculated with the unfiltered serum, was slightly sick on the seventh, eighth, and ninth days after inoculation, but recovered entirely and was immune when subsequently placed in exposure pen;

in this case it would seem probable that the animal possessed a partial immunity prior to inoculation, and that this immunity was sufficiently heightened by the inoculation of the unfiltered serum to render the animal completely immune when placed in the exposure pen; this supposition is justified by the fact that the animal was made only slightly sick by the inoculation of unfiltered serum.

Hogs 195 and 196, inoculated with the filtered serum, were both sick on the ninth day after inoculation and continued quite sick for 2 weeks; these animals did not recover fully from the effects of the inoculation, but remained thin and runty; when exposed subsequently both animals proved to be immune. In this instance it would seem that the immunity was a result of the inoculation with the filtered serum.

The failure of hog 197 to become sick when exposed to the sick filtered-serum hogs can not be explained on the ground of natural immunity, for the animal died promptly when placed in the exposure pen. A possible explanation may be the low degree of virulence of the blood used for the injections, though we are not prepared to state that the contagiousness depends upon the virulence. The result in this case is so exceptional in our experience that a fully satisfactory explanation does not occur to us.

In this experiment it is also rather difficult to explain why the serum was not more virulent, as the animal from which the blood was drawn exhibited well-marked lesions of acute, hemorrhagic hog cholera, and the mortality in the herd from which the blood was obtained was quite high; 94 per cent of the entire herd were sick and 90 per cent died.

EXPERIMENTS WITH BLOOD FROM HERD VIII.

This herd consisted originally, at the time of the outbreak, July 28, 1904, of 536 medium-sized hogs, but on August 12, 1904, the date on which the material was obtained for the experiments described below, 136 hogs had died, and many of the animals showed large ulcers on various parts of the body; but coughing and diarrhea were not prominent symptoms. The final report on November 2, 1904, gave a total loss of 310 hogs and the rest as all thrifty. The fact that this herd contained no suckling pigs and very few shoats weighing less than 100 pounds accounts, in all probability, for the greater number surviving in this as compared with other herds. Three animals were selected for autopsy, as follows:

Hog 1: The animal weighed about 150 pounds; died in convulsions; skin very yellow; inguinal glands enlarged and slightly hyperemic; lungs slightly congested, with areas of consolidation; spleen much enlarged, dark, and soft; kidneys dark in color and somewhat mottled; mucosa of large intestine showed patches of congestion and a small yellow ulcer at the extreme end of the cecum. No cultures were made from this animal.

Hog 2: A small shoat, weight about 60 pounds, very sick and scarcely able to stand. There were two large raw ulcers on the hips, and the animal exhibited diar-

rhea. Inguinal glands and lymphatics generally red and swollen; small hemorrhagic patches in the lungs; spleen enlarged and dark; scattered punctate hemorrhages in the kidneys; large intestine showed numerous large ulcerations in the mucosa. No cultures were made from this animal.

Hog 3: An animal weighing about 100 pounds; was etherized on August 2, 1904, and the heart blood was drawn and used in Filtration Experiment No. 23. The autopsy on this animal showed inguinal glands enlarged and hyperemic; few scattered areas of congestion in the lungs; liver dark and rather soft; spleen considerably enlarged, dark, and quite soft; right kidney congested and rather pale; left kidney congested, with large necrotic areas, which had the appearance of multiple abscesses filled with dry, inspissated pus; suprarenal gland on left side had the appearance of a mass of large thick-walled, convoluted tubules; mucosa of stomach showed patches of congestion toward the pylorus; cecum and large intestine showed patches of thick, dirty-white exudate, probably the site of beginning ulcerations. Cultures from heart blood and spleen failed to show *B. cholerae suis*.

FILTRATION EXPERIMENT No. 23.

The heart blood of hog 3, herd VIII, drawn by means of a sterile canula, was defibrinated, filtered through absorbent cotton, and placed on ice overnight. At the end of 18 hours, when the red blood corpuscles had settled out, the serum was drawn off with a sterile pipette. To 50 c. c. of the clear serum, 500 c. c. of neutral beef broth and 3 c. c. of a 24-hour bouillon culture of *B. prodigiosus* were added, and of this mixture 250 c. c. was passed through a Chamberland "B" cylinder.

Of the diluted serum, before filtration and before the addition of *B. prodigiosus*, 25 c. c. was set aside for the inoculation of a hog, and a like amount was added to a flask of sterile bouillon (200 c. c.) but failed to show *B. cholerae suis*.

A portion of the diluted filtered serum was drawn off with a sterile pipette for the inoculation of hogs, and the remainder set aside in the dark. At the end of 48 hours this filtrate showed clouding, and agar plates made therefrom showed (1) a small micrococcus forming lemon-yellow colonies, and (2) a large heavy bacillus, but no colonies of *B. cholerae suis* or *B. prodigiosus*.

The following is a list of the animals inoculated, with brief clinical notes and autopsy records in each case:

Hog 203 was inoculated with 25 c. c. of diluted unfiltered serum of hog 3; showed first symptoms of sickness 5 days after inoculation, became rapidly worse, and died 11 days after receiving the injection. Inguinal glands were red and swollen; lungs much congested, and pleural cavity contained a sticky, serofibrinous exudate; coronary vessels engorged and pericardial sac distended, with a bloody, serofibrinous exudate; liver much congested; spleen very dark and soft; kidneys congested and thickly studded with small, punctate hemorrhages; mucosa of cecum and colon slightly congested; mesenteric glands red and swollen. Agar cultures from heart blood and spleen showed pure growths of *B. cholerae suis*.

Hog 201 was inoculated with 25 c. c. of diluted filtered serum of hog 3; showed first symptoms of sickness 8 days after inoculation; became rapidly worse, and died 13 days after receiving the injection of diluted serum. Autopsy showed the inguinal glands on one side slightly reddened; a few small, hemorrhagic patches in lungs; liver dark and mottled; spleen enlarged, dark, and soft, and thickly studded with large, reddish elevations that gave the organ a rough, coarsely granular appearance; kidneys congested, with a few scattered, punctate hemorrhages; mucosa of stomach intensely congested toward pylorus; entire mucosa of cecum ulcerated and covered with thick, yellowish white, necrotic exudate; mucosa of large intestine intensely reddened, with evidences of beginning ulceration; mesenteric glands red and swollen. Agar cultures from heart blood and spleen showed pure growths of *B. cholerae suis*.

Hog 202 was inoculated in the same manner as hog 201; showed first symptoms of sickness 8 days after inoculation; became gradually worse, and was etherized when in a dying condition 15 days after receiving the injection of filtered serum, in order to obtain blood for another experiment. Autopsy showed inguinal glands red and swollen; numerous small, scattered areas of congestion in the lungs; liver dark red and firm; spleen greatly enlarged, very dark, and very soft; kidneys congested and thickly sprinkled with small, dark, hemorrhagic points; small hemorrhagic points on auricular appendages of heart; marked congestion of mucosa of stomach toward pylorus; mucosa of cecum and colon congested, with several small but well-defined ulcers near the ileocecal valve; mesenteric glands red and swollen. Agar cultures from heart blood and spleen showed pure growths of *B. cholerae suis*.

Hog 200 was injected subcutaneously with 25 c. c. of sterile bouillon and placed in the pen with hogs 201 and 202; was first noticed sick 17 days after exposure to these hogs, became rapidly worse, and died 3 days later. Autopsy showed enlarged inguinal glands; lungs congested and filled with frothy serum; small hemorrhagic patches on auricular appendages of heart and considerable serofibrinous exudate in pericardial sac; liver much congested; spleen enlarged, dark, and soft, with large, reddish elevations that gave the organ a roughened appearance; kidneys congested, with a few punctate hemorrhages; mucosa of stomach considerably congested toward pylorus; hemorrhagic patches in cecum, but no ulcers. Agar cultures from heart blood and spleen showed pure growths of *B. cholerae suis*.

TABLE 37.

No. of hog.	Material injected.	Inoculation.		Autopsy.	Remarks.
		Date.	Result.		
203	25 c. c. unfiltered serum hog 3, herd VIII.	1904. Aug. 13	1904. Sick Aug. 18; died Aug. 24.	Hemorrhages.	<i>B. cholerae suis</i> present.
201	25 c. c. filtered serum hog 3, herd VIII.	do....	Sick Aug. 21; died Aug. 26.	Hemorrhages and ulcers.	Do.
202	do.....	do....	Sick Aug. 21; killed Aug. 28.	do.....	Do.
200	25 c. c. sterile bouillon; check, exposed to hogs 201 and 202.	do....	Sick Aug. 30; died Sept. 2.	Hemorrhages.	Do.

The results in this experiment are in harmony with those obtained in other cases. The hogs inoculated with the filtered serum both became sick on the same day, and the check hog contracted the disease by association with these animals, becoming sick 9 days later than the inoculated animals. The hog injected with the unfiltered blood became sick 2 or 3 days sooner than the hogs injected with the filtered blood, and the length of time between the appearance of the first symptoms and death was longer in one of the filtered serum hogs than in the unfiltered serum hog, but shorter in the other. *B. cholerae suis* was present in all the hogs concerned in the experiment, though it was absent from the filtered serum as well as from the unfiltered serum.

FILTRATION EXPERIMENT NO. 24.

The blood used in this experiment was obtained from hog 202, which was inoculated with 25 c. c. of the diluted filtered serum from hog 3. (See Filtration Experiment No. 23.)

The heart blood of hog 202, drawn by means of sterile canula, was defibrinated, filtered through absorbent cotton, and allowed to stand on ice for 26 hours in order to give the red blood corpuscles time to settle out. To 25 c. c. of the clear serum, free from red blood cells,

was then added 250 c. c. of neutral beef broth and 3 c. c. of a 24-hour bouillon culture of *B. prodigiosus*, and the mixture passed through a Chamberland "B" cylinder. A portion of the diluted serum, before filtration and before the addition of *B. prodigiosus*, was used for the inoculation of a hog, and cultures made from the diluted serum previous to filtration showed *B. cholerae suis*. The clear filtrate was set away in the dark and allowed to stand at room temperature (about 25° C.) for 3 days, at the end of which time it remained perfectly clear, and was then used for the inoculation of experiment animals.

The following is a list of the animals used in this experiment, with brief clinical notes and autopsy records in each case:

Hog 215 was injected subcutaneously with 25 c. c. of diluted unfiltered serum of hog 202; was first noticed sick 3 days after inoculation, became rapidly worse, and died 8 days after receiving the injection. Autopsy showed slight reddening of integument in pubic and thoracic regions; inguinal glands red and swollen; large areas of congestion in lungs; liver dark and soft, apparently engorged with blood; spleen enlarged, dark, and soft; kidneys much congested and thickly studded with small punctate hemorrhages; small hemorrhagic patches in mucosa of cecum. Agar cultures from heart blood and spleen showed pure growths of *B. cholerae suis*.

Hog 222 was injected subcutaneously with 25 c. c. of the diluted filtered serum; was first noticed sick 6 days after inoculation, became gradually worse, and died 19 days after receiving the injection. Autopsy showed inguinal glands greatly enlarged and very dark red; lungs congested, with small hemorrhagic patches; liver dark and full of blood; spleen enlarged, dark, and very soft; kidneys congested, with a few scattered punctate hemorrhages; several large hemorrhagic patches in mucosa of cecum; mesenteric and lumbar glands red and swollen. Agar cultures from heart blood and spleen failed to show *B. cholerae suis*.

Hog 223 was injected in the same manner as hog 222; was first noticed sick 10 days after inoculation, became gradually worse, and died 23 days after receiving the injection. Autopsy showed inguinal glands somewhat enlarged and red; small patches of congestion in the lungs; liver dark and full of blood; spleen greatly enlarged, dark, and very soft; kidneys pale and mottled; mucosa of stomach very red; small, early ulcerations covered with thick, yellow exudate in cecum; mesenteric and lumbar glands congested. Agar cultures from heart blood and spleen failed to show *B. cholerae suis*.

Hog 224 was not inoculated, but exposed in pen with hogs 222 and 223; was first noticed sick 16 days after being placed with hogs 222 and 223; became gradually worse, and died 28 days later. Autopsy showed the usual lesions of chronic hog cholera. No cultures were made.

TABLE 38.

No. of hog.	Material injected.	Inoculation.		Autopsy.	Remarks.
		Date.	Result.		
215	25 c. c. unfiltered serum hog 202.	1904. Aug. 29	1904. Sick Sept. 1; died Sept. 6.	Hemorrhages.	<i>B. cholerae suis</i> present.
222	25 c. c. filtered serum hog 202.	Sept. 2	Sick Sept. 8; died Sept. 21.	do.....	<i>B. cholerae suis</i> not present.
223	do.....	do.....	Sick Sept. 12; died Sept. 25.	Hemorrhages and ulcers.	Do.
224	Check; exposed to hogs 222 and 223.	(a)	Sick Sept. 18; died Oct. 1.	Ulcers.....	No cultures taken.

a Exposed September 2.

In this experiment the hogs inoculated with filtered serum died with characteristic lesions, and the contagiousness of the disease produced by the filtered serum is shown by the record of the pen check which became sick 6 days after the last of the 2 inoculated hogs became sick.

B. cholerae suis could not be detected in the cultures made from the filtered-serum animals. The hogs inoculated with filtered serum in this experiment became sick and died later than the hog inoculated with unfiltered serum, and this may have been due to a portion of the virus having been removed by the filtration; but as hogs inoculated with unfiltered blood have not always been the first to succumb, we are not fully convinced that this was the cause of the slower progress of the disease in the filtered-serum hogs in this case.

The virus used in this experiment possesses special interest from the fact that the hog furnishing the blood was inoculated with filtered serum from a hog that had been inoculated with unfiltered blood which did not contain *B. cholerae suis*.

DISCUSSION OF FILTRATION EXPERIMENTS.

The results of the subcutaneous injections of hog cholera blood filtered through Chamberland and Berkefeld cylinders show that these injections produced disease quite as uniformly as those with the unfiltered blood. On the whole, it is true, hogs inoculated with the unfiltered blood became sick more quickly after inoculation than the hogs inoculated with the same serum after filtration, but this was not always the case. It is also true that a greater percentage of deaths occurred among the hogs inoculated with the unfiltered serum than among the hogs inoculated with the filtered serum. Of the 36 hogs inoculated with the unfiltered serum, all became sick, 24 died, 10 were killed in a moribund condition, and 2 recovered; while of the 61 hogs injected with filtered serum, 58 became sick, 39 died, 10 were killed in a moribund condition, 9 recovered, and 3 showed no perceptible sickness. So although the mortality was less in the hogs injected with filtered serum, there was practically no difference in the production of disease in the case of unfiltered and of filtered blood; for the 3 hogs which failed to show noticeable symptoms of sickness after the injection of filtered blood must have possessed great resisting power, since 2 of them remained well on exposure to natural infection, and the other lived for many weeks in contact with sick hogs but died eventually, though it is not certain that death was due to hog cholera.

The resisting power of these 3 hogs may have been natural, or, as will appear from what is said later on, it may have been acquired from the serum injections. The possibility that they may have been really sick, but not have shown visible evidences of this, should not be lost sight of.

It is not surprising, however, that the blood after filtration should show less potency than blood before filtration; for, whatever the nature of the infectious agent, it is but natural to suppose that some of it is removed by the filter. Nevertheless, diminution of potency is

not observed in all cases, as already remarked, and when no diminution is observed it is probable that there is such a large amount of the infectious agent present originally, that although a part is removed by filtration, there still remains a sufficient amount to cause disease.

It will be observed that these results show not only that the filtered blood produces disease with great regularity, but that hogs which recover from the disease produced in this way have immunity from natural infection. Of the 9 hogs that recovered after being sick, only 1 died in the exposure pen, but from the history of this case it seems probable that this hog, 1137, may not have fully recovered from the sickness produced by the filtered serum.

The results furthermore show that the disease produced by injection of filtered blood is contagious; for uninoculated hogs placed in the pens with the hogs inoculated with the filtered blood remained well until the inoculated animals became sick, and then contracted disease more or less promptly. There were 22 such check hogs in the pens with the hogs inoculated with filtered serum, and 17 of the 22 contracted the disease, while 5 did not show noticeable symptoms of illness.

Of the 17 that became sick, 14 died, 2 were killed in a moribund condition, and 1 recovered and remained well on exposure to natural infection. Of the 5 which showed no disease from association, 1 remained well in the exposure pen and the others died. So the disease produced by the filtered blood proved contagious in at least 77 per cent of cases, and four of the five failures may possibly be accounted for by the fact, which can be verified from the records, that the disease in the inoculated animals to which these checks were exposed was of a mild type. The other failure was probably a case of natural immunity, since the hog did not contract the disease on exposure to natural infection.

The experiments made to test the infectiousness of the blood and tissues of animals sick as a result of the inoculation of filtered blood show that the blood of such animals is infectious and that the viscera cause the disease on feeding. The urine was tested and found to be infectious in one case. This urine contained *B. cholerae suis*, but, since this organism does not appear to affect the virulence of the blood, it could hardly be regarded as the cause of the pathogenic property of the urine.

It is evident that there is no essential difference between the disease produced by filtered diseased blood, on the one hand, and that acquired in natural outbreaks, on the other. The symptoms and lesions differ in no particular in the one case from those in the other; but still more striking and convincing evidence of identity is the fact that hogs recovered from sickness produced by the filtered blood have immunity from the natural disease.

We would emphasize the fact that the filtered blood was shown to be free from *B. cholerae suis* by culture tests in all cases, and by the inoculation of rabbits or guinea pigs in most cases.

GENERAL CONCLUSIONS.

We have called attention to the fact that acute hog cholera, at least the form of that disease encountered in southwestern Iowa, possesses, in addition to those symptoms and lesions generally recognized, certain other essential features which have not been considered as fully in previous work as, in our opinion, their importance deserves. These features upon which we wish to lay especial stress are:

(1) Contagiousness: The disease should be communicable from sick to well animals by association. (2) Infectiousness: The blood of sick animals should cause disease upon subcutaneous injection into healthy nonimmunes. (3) Immunity: After recovery the animals should resist subsequent exposure to the disease.

Our experiments have shown that pure cultures of *B. cholerae suis* injected subcutaneously into hogs usually produce but slight disturbance, although after intravenous injections or feeding a severe illness frequently results. The disease produced in this manner may present the symptoms and lesions seen in acute hog cholera, but does not possess the characteristics of contagiousness nor of infectiousness of the blood; nor are those hogs which have recovered from such illness immune when exposed subsequently to the natural disease. In regard to the experiments with pure cultures of *B. cholerae suis*, therefore, we may say that they demonstrate the very considerable pathogenic power which that organism possesses for hogs, but they also show that the disease produced by that organism lacks several of the essential features of acute hog cholera.

The experiments with blood serum derived from hogs sick of hog cholera and proven to be free from *B. cholerae suis* show, on the contrary, that such serum produces illness in hogs with great regularity upon subcutaneous injection, and, furthermore, that the disease thus produced possesses all of the characteristics of the natural disease, including symptoms, lesions, contagiousness, infectiousness of the blood, and immunity in those animals which recover. These results are in such striking contrast to those obtained when cultures of *B. cholerae suis* are used, and they are in such perfect harmony with those obtained by the use of unfiltered blood from sick hogs, that we are forced to conclude that there exists in the blood of hogs suffering from acute hog cholera some virus other than *B. cholerae suis*, and that this virus is necessary for the production of that disease.

This virus, present in the blood of hogs sick of acute hog cholera, but absent from pure cultures of *B. cholerae suis*, is known to us only

by the effects it produces. We have failed completely in all attempts to discover by microscopic examination or by the usual cultural methods any visible microorganism in these filtrates. That the pathogenic power of the filtered blood is due to some living agent endowed with the power of reproduction, and not to the presence of a toxine alone, there can be no doubt, from the fact that the disease induced by the filtered serum is communicated from sick to healthy animals by association, and, moreover, from the fact that we have transferred the disease induced by filtered serum to a second and even a third animal by subcutaneous injections, the serum being filtered each time previous to inoculation.

While our experiments establish beyond question that the filterable virus was present in all the outbreaks of hog cholera studied experimentally by us, it is also true that *B. cholerae suis* was present almost as uniformly. So it would be impossible to overlook, even if we were inclined to do so, the part which this organism may have played. But from the results of our inoculations with the filterable virus, on the one hand, and our results and those of others with cultures, on the other, we are compelled to conclude that the prime cause in our cases was the filterable virus, and that *B. cholerae suis* was at most an accessory factor.

The exact rôle played by *B. cholerae suis* in outbreaks of acute hog cholera is difficult to define. That the fatal result in many instances is materially influenced by the presence of that organism can not be doubted, and, in addition, we would emphasize the fact that although the filterable virus appears to have been the primary invader, in those cases of acute hog cholera which we have studied, we do not deny the possibility of independent disease being caused by *B. cholerae suis*. In fact it is difficult to avoid a belief in such a possibility, on account of the very considerable pathogenic power for hogs exhibited by many cultures of that organism when fed or administered intravenously. The filterable virus seems not only to cause disease by itself, as shown by the results of many of our experiments, but it seems also to be able to lower the resisting power of the hog and thus enable *B. cholerae suis* to invade the body, and this we believe takes place in the majority of cases in natural outbreaks. If the filterable virus is capable of lowering the resisting power of hogs for *B. cholerae suis*, then it is easily conceivable that other agencies may also lessen the resisting power of these animals.

So it must be admitted that a disease in hogs may exist which is due to *B. cholerae suis*, and having no connection with the filterable virus found by us in the outbreaks we have studied. From the knowledge we have of the pathogenic powers of *B. cholerae suis*, however, we would expect that any disease in which it is the chief pathogenic factor would be possessed of a low degree of contagiousness.

The fact that *B. cholerae suis* was found in a large proportion of the cultures taken from hogs inoculated with filtered serum seems to

indicate strongly that that organism has its normal habitat on or in the bodies of healthy hogs, and that in the case of lowered resistance on the part of the hogs this organism enters the circulation. A careful study of the bacterial flora of the hog's alimentary tract might throw considerable light upon this subject.

We have begun investigations of this nature, but as yet other more pressing work has prevented their completion. In a few preliminary examinations, guinea pigs and rabbits were inoculated subcutaneously with small amounts of the intestinal contents of normal hogs and a few agar plates were made from the same material. In one case only, when the method of Drigalski and Conradi for the isolation of typhoid bacilli was used, did we succeed in discovering an organism which possessed the cultural characters of *B. cholerae suis*. The only way in which this organism from the normal intestinal contents differed from the classical type of *B. cholerae suis* was in its lack of pathogenic power for guinea pigs. It will be readily understood that the isolation of *B. cholerae suis* from material containing an immense number of colon bacilli is a matter of unusual difficulty, and it is therefore not surprising that we should fail to discover it even though it may have been present in very considerable numbers. The isolation of a non-virulent form of *B. cholerae suis* from the intestines is, therefore, considered very important on account of the support it lends to the hypothesis that *B. cholerae suis* may be frequently an inhabitant of the intestine of the normal hog.

If such a hypothesis be true, the conditions would be entirely analogous to those under which many pathogenic microorganisms exist, as, for example, the pneumococcus in the mouths of healthy individuals, and the swine plague bacillus (*B. suis septicus*) on the tonsils of healthy hogs, and in the pens where healthy hogs are kept, the supposition being that microorganisms present in small numbers or possessing a low degree of virulence are successfully resisted until certain influences either heighten the virulence of the bacteria or lower the resisting power of the animal body. The production of disease by feeding small quantities of *B. cholerae suis* might seem to indicate that that organism could not remain in the hog's intestine without giving rise to more or less serious disturbance, but it should be remembered that these small quantities of culture ($\frac{1}{10}$ to $\frac{1}{2}$ c. c.) contain enormous numbers of bacilli and probably many more than the hog under ordinary circumstances is able to resist.

The toxin present in artificial cultures may also play some part in causing illness when cultures are fed, or it may be—and this last supposition seems not unlikely—that *B. cholerae suis*, if it exists in the bodies of normal hogs, possesses a very low degree of virulence, and that it is only through a lowered resistance on the part of the hog that it is enabled to enter the circulation and cause disease. The

isolation from a healthy hog of an organism indistinguishable from *B. cholerae suis* except by absence of pathogenic power for guinea pigs lends strength to the explanation last mentioned of the way in which hogs are liable to attacks by *B. cholerae suis*. *B. coli communis* is a familiar example of an organism constantly present in health, and yet assuming under certain conditions very great pathogenic power.

Whatever may be the port of entry or the influences which bring about an invasion by *B. cholerae suis*, we are convinced that the filterable virus was responsible for the high degree of infectiousness and, therefore, for the spread of the disease encountered in the several outbreaks of acute hog cholera described in previous pages of this bulletin. It seems reasonable to suppose that what has been found to be true in these eight outbreaks is also true of other epidemics of acute hog cholera, and therefore that the extensive losses occasioned by outbreaks of that disease are to be attributed, in the main, to the filterable virus. In many of the individual hogs the fatal termination may have been in large measure due to *B. cholerae suis*, but the probabilities are that, without the filterable virus, comparatively few hogs would have been attacked.

It follows from all that has been demonstrated in the preceding pages that, if a practical method of protecting hogs from the filterable virus should be discovered, the problem of combating hog cholera, at least the highly infectious form of that disease, will have been solved.